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THE UNIVERSITY OF ALBERTA

STRUCTURAL AND SYNTHETIC STUDIES ON NATURAL PRODUCTS

Part (1) An approach to the Iridoid monoterpenes.

Part (2) Some transformations of an annotinine derivative.

Part (3) The structure of Cyathic acid.

by

© RICHARD J. FLANAGAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSPHY

IN

CHEMISTRY

EDMONTON, ALBERTA

Autumn 1978

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled STRUCTURAL AND SYNTHETIC STUDIES ON NATURAL PRODUCTS submitted by Richard J. Flanagan in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry.

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J. Hooz

J.W. Lown

J. Takats

R.T. Coutts

A.G. Fallis, External Examiner

Date 25th September 1978

TO MAUREEN -

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Some other Man, in my Place, would perchance, make you twenty Apologies, for his want of Skill, and Address, in governing this Affair, but these are *Formal*, and *Pedantique Fooleries:* As if any Man that first takes himself for a Coxcomb in his own Heart, would afterwards make himself one in Print too. This *Abstract*, such as it is, you are extremely welcome to; and I am sorry it is no better, both for your sakes and my own.

Books, and Dishes have this Common Fate; there was never any One, of Either of them, that pleas'd All Palates. And, in Truth, it is a Thing as little to be *Wish'd for*, as *Expected*; For, an Universal Applause is at least Two Thirds of a Scandal. So that though I deliver up these Papers to the Press, I invite no Man to the Reading of them: And, whosoever Reads, and Repents; it is his Own Fault. To Conclude, as I made this Composition Principally for my Self, so it agrees exceedingly Well with My Constitution; and yet, if any Man has a Mind to take part with me, he has Free Leave, and Welcome.

Sir Roger L'Estrange in 1673

ABSTRACT

Part (1) describes several attempts to generate the iridoid skeleton from 10-functionalized geraniol derivatives. Several electrophilic, nucleophilic and photochemical reactions were investigated. As a result of some observations on the nature of allylic Grignard reagents a synthesis of an isomer of farnesol was completed.

In part (2) a successful chemical correlation between the annotinine and the ester "M" skeleton was achieved. This correlation took advantage of the highly strained nature of the cyclopropane ring in ester "M" and its successful cleavage using lithium in ammonia. The carbon-13 magnetic spectra of a number of annotinine and ester "M" derivatives were measured and the various absorptions assigned.

Part (3) is an account of the various chemical and physical methods by which the structure of cyathic acid, a new novel pentacyclic triterpene acid, was determined. This new acid was correlated with the known co-occurring compounds glochidone and glochidonol. The carbon-13 magnetic resonance spectra of a large number of derivatives of cyathic acid, glochidone, glochindonol and lupeol were measured and assigned.

ACKNOWLEDGEMENTS

I would like to thank:

My father who took it on faith that chemistry was a fitting endeavour,

My mother whose interest and care are always with me,

Dr. William Ayer whose guiding hand permeates this work, whose tolerance knows no bounds and who came through with financial assistance.

Jacki Jorgensen who typed my manuscript and fielded red tape for me,

John Mercer, my true friend, a rare find,

Sheldon and Freddie who took a strong interest in my thesis,

Maureen, my wife, whose rare insight and encouragement persuaded me to retrace my old steps.

Mother nature, the provider of these tormenting molecules and whose perverse sense of humour is everpresent.

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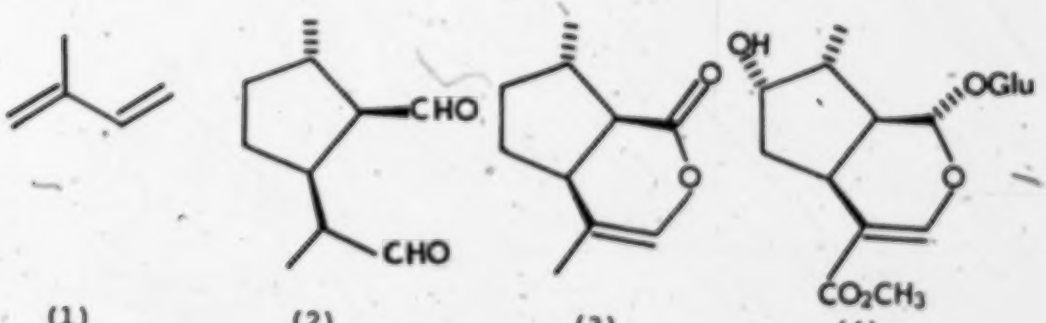
AN APPROACH TO THE IRIDOID MONOTERPENES

CHAPTER ONE

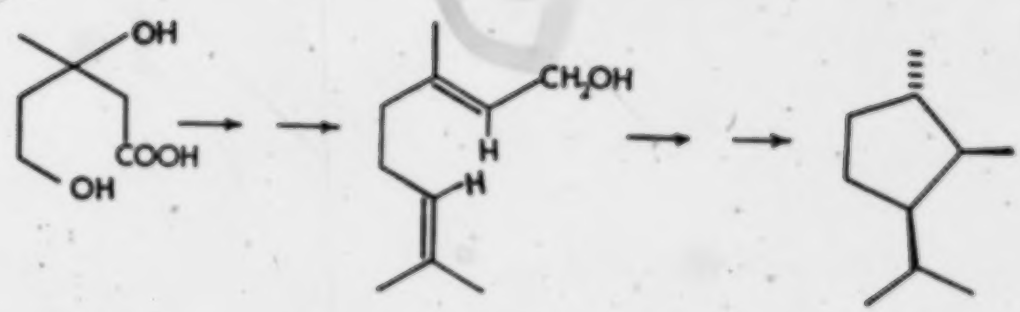
INTRODUCTION

Since the dawn of organic chemistry many naturally occuring compounds have been discovered. Among these are the class of monoterpenes. Monoterpenes may be regarded as formed by the union of two molecules of isoprene (1) and therefore contain ten carbon atoms. This is their distinguishing characteristic. A particular type of monoterpene is the Iridoids. These are ten carbon molecules which contain a five-membered ring. Some examples of these are irododial[1] (2) nepetalactone[2] (3) and loganin[3] (4).



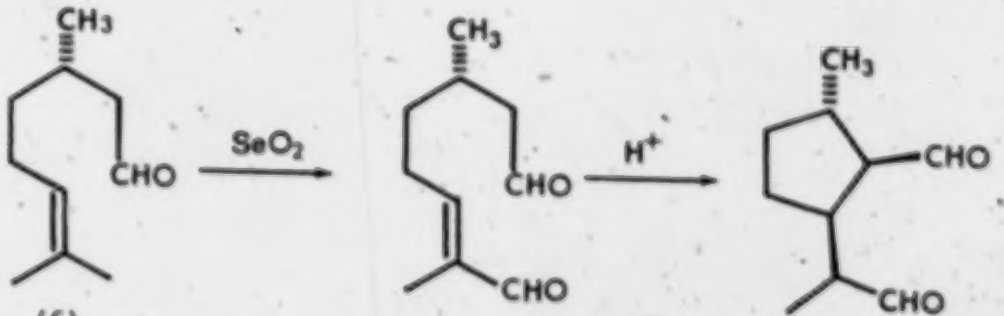
(1) (2) (3) (4)

The stereochemistry (5A) found in these molecules is common to nearly all members of the Iridoids. It is reasonable therefore to assume that they are all produced by the same biosynthetic route. Since they are obviously monoterpenes it is further assumed that they are derived from geraniol (5) and hence from mevalonic acid (4A).



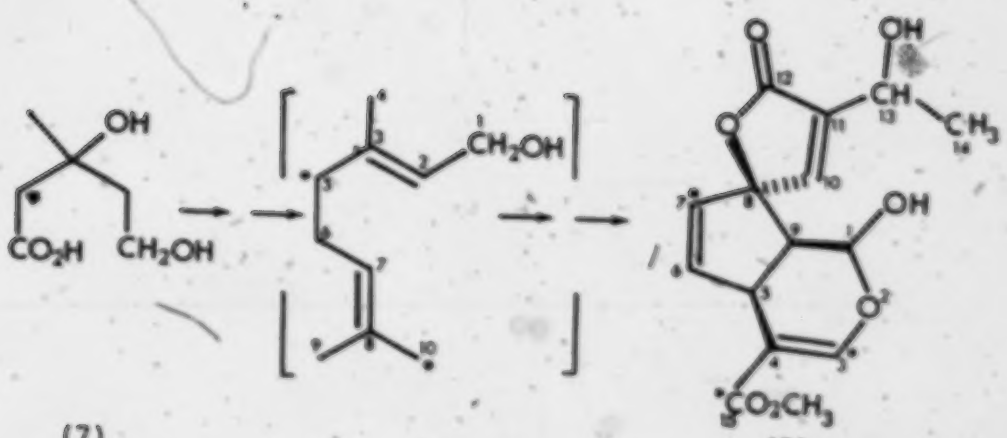
(4A) (5) (5A)

In 1957 Sir Robert Robinson suggested that the Iridoids were derived from geraniol or a derivative thereof.[4] He showed the feasability of his idea by synthesizing irododial (2) from (-) citronellal (6).



(6) (2)

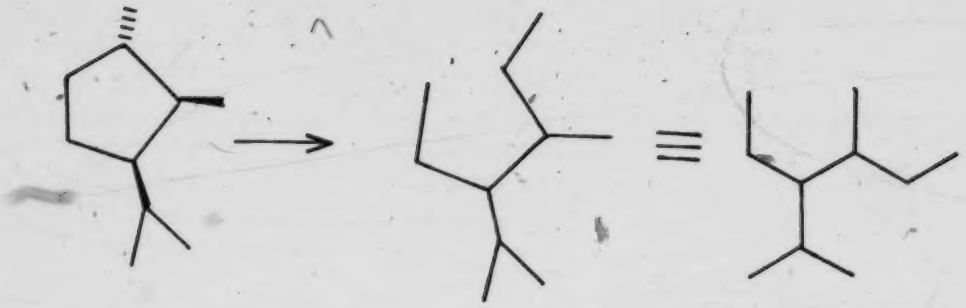
The earlier part of the biosynthetic pathway was revealed by the use of radioactive tracers. Thus in 1964 Yeowell and Schmid administered sodium 2-^{14}C mevalonate (7) to the leaves of *Plumeria acutifolia* and found radioactivity in the aglucone of the plumieride[5] (8).



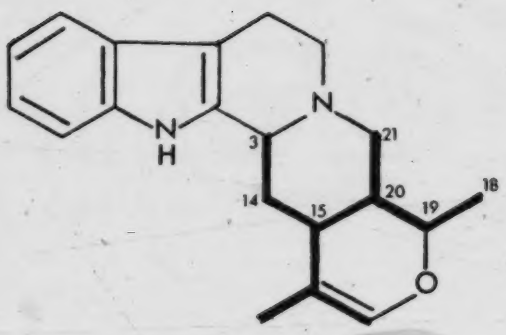
(7) (8)

Interestingly, half of the total radioactivity was equally divided between carbons 3 and 15 of (8) and the other half was found to be on carbons 5, 6 or 7. This implies that somewhere on the route from geraniol to plumieride, carbons 9 and 10 of geraniol became equivalent.

Further developments in iridoid biosynthesis were brought about indirectly as a result of work done on indole alkaloid biosynthesis. In 1961 Thomas suggested that the non-tryptamine part of the indole alkaloids was derived from an iridoid monoterpene[6] (5A). Thus in ajmalicine (9) the heavily outlined carbon atoms are equivalent to a cleaved iridoid (10).

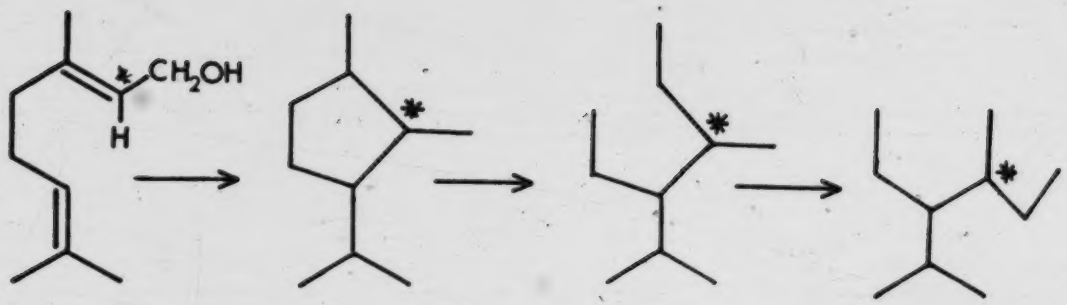


(5A) (10) (10)



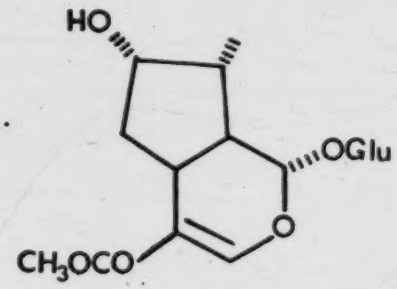
(9)

In 1966 both Battersby and Arigoni showed that 2-^{14}C geraniol (11) was incorporated into ajmalicine (9) and that the radioactivity was present at C-20.[7]



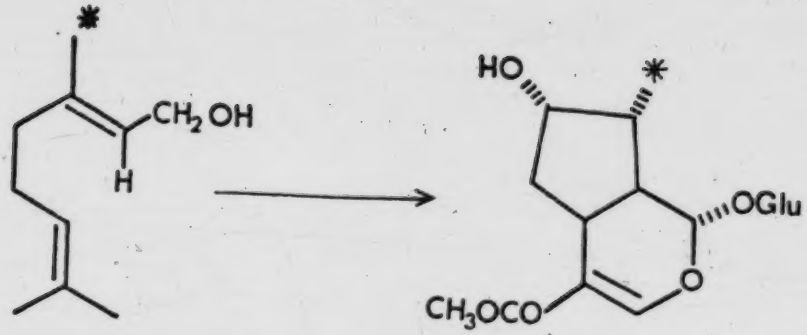
(11)

Later the same year Battersby suggested that the intermediate iridoid involved in indole alkaloid biosynthesis was loganin[8] (4).



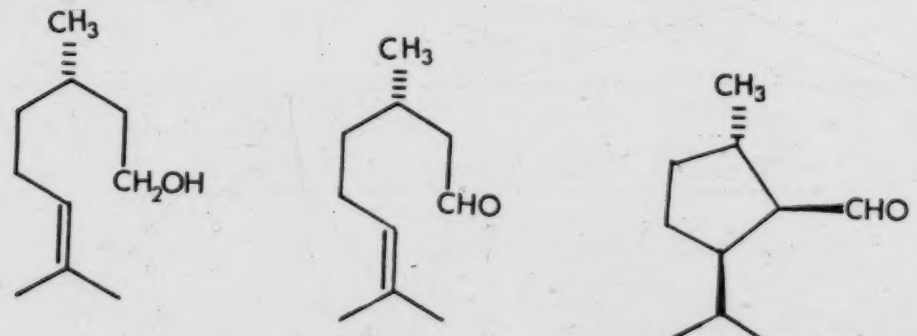
(4)

This was proven in 1968 when Arigoni, using the rhizomes of *Menyanthes trifoliata*, showed that 4-^{14}C geraniol (13) gave rise to loganin (4) containing 85% of the activity in the C-3 methyl group.[9]



(13) (4)

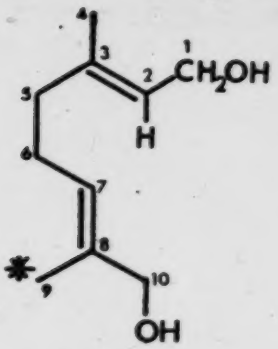
Thus the link between geraniol and the iridoid monoterpenes was finally established. It has been suggested that citronellol (14), citronellal (6) and irododial (2) were intermediates on the route from geraniol to loganin but this was discounted by Bowman in 1969 when he showed that irododial is not incorporated in the indole alkaloids of *Vinca rosea*.[10]



(14) (6) (2)

In 1970 Battersby and Arigoni both showed that 10-hydroxygeraniol (15) and 10-hydroxyneral (16) were

efficient precursors of loganin[11]



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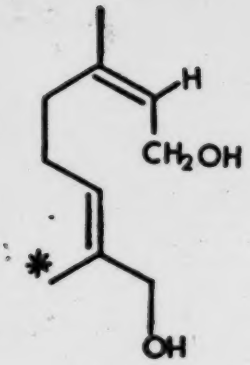
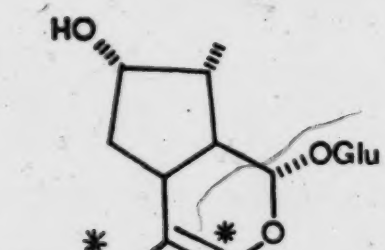


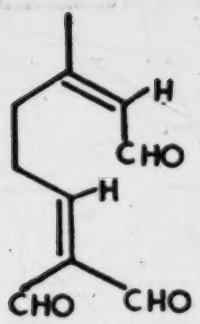
(16)

They also found however that (9-C^{14}) 10-hydroxy geraniol and (9-C^{14}) 10-hydroxy nerol gave rise to loganin (4) in which the label is equally divided between C-3 and C-11.



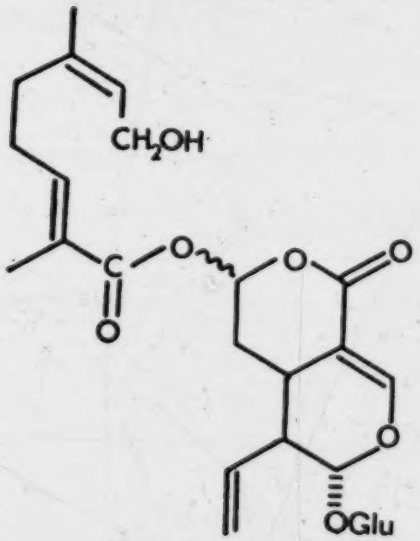
(4)

They therefore postulated the intermediacy of the trialdehyde (17) between geraniol and loganin.



(17)

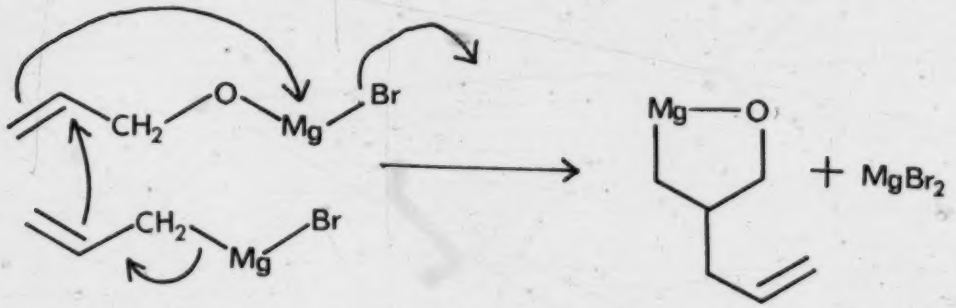
They chose the neral version of the trialdehyde rather than the geranial version on account of the co-occurence of foliamenthin (18) in similar plants.[12]



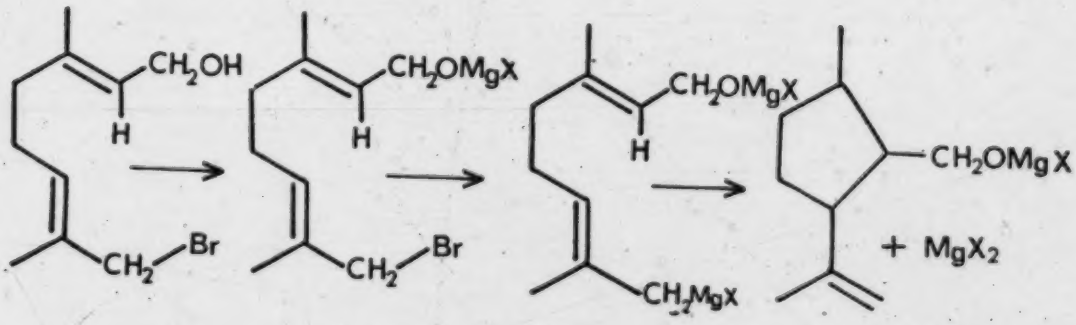
(18)

We were encouraged by all this work to look for a new biomimetic approach to the synthesis of Iridoids. In particular the discovery in nature of 10-functionalized

geraniol derivatives led us to believe that we could form the cyclopentane ring by simply introducing a single functionality at C-10 in geraniol. This was based on the earlier discovery that allylic Grignard reagents could add to the double bond of the salts of certain allylic alcohols,[13] e.g.,



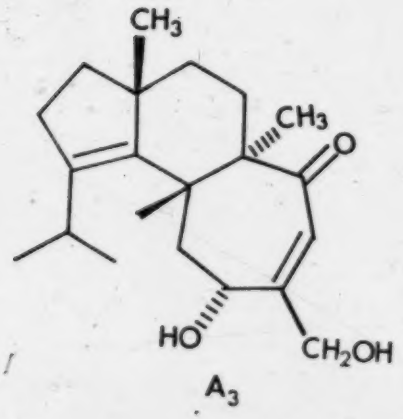
We felt a similar reaction might occur with the Grignard derivative of 10-bromogeraniol



The stimulus for our search for a new biometic route to the iridoids was provided by the discovery in these

laboratories of a new class of diterpenes which contained similar structural characteristics to those found in the Iridoids.[14]

These are the cyathins - a group of tricyclic diterpenes containing five, six and seven-membered rings. They are obtained from *Cyathus helenae* - a bird's nest fungus which grows at an altitude of about 7000 feet in the Canadian Rockies. Two examples of cyathins are A_3 (19) and B_3 (20).



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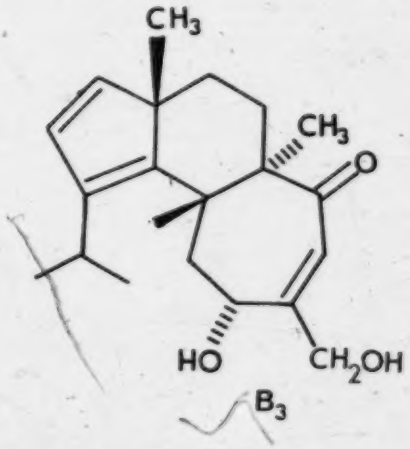
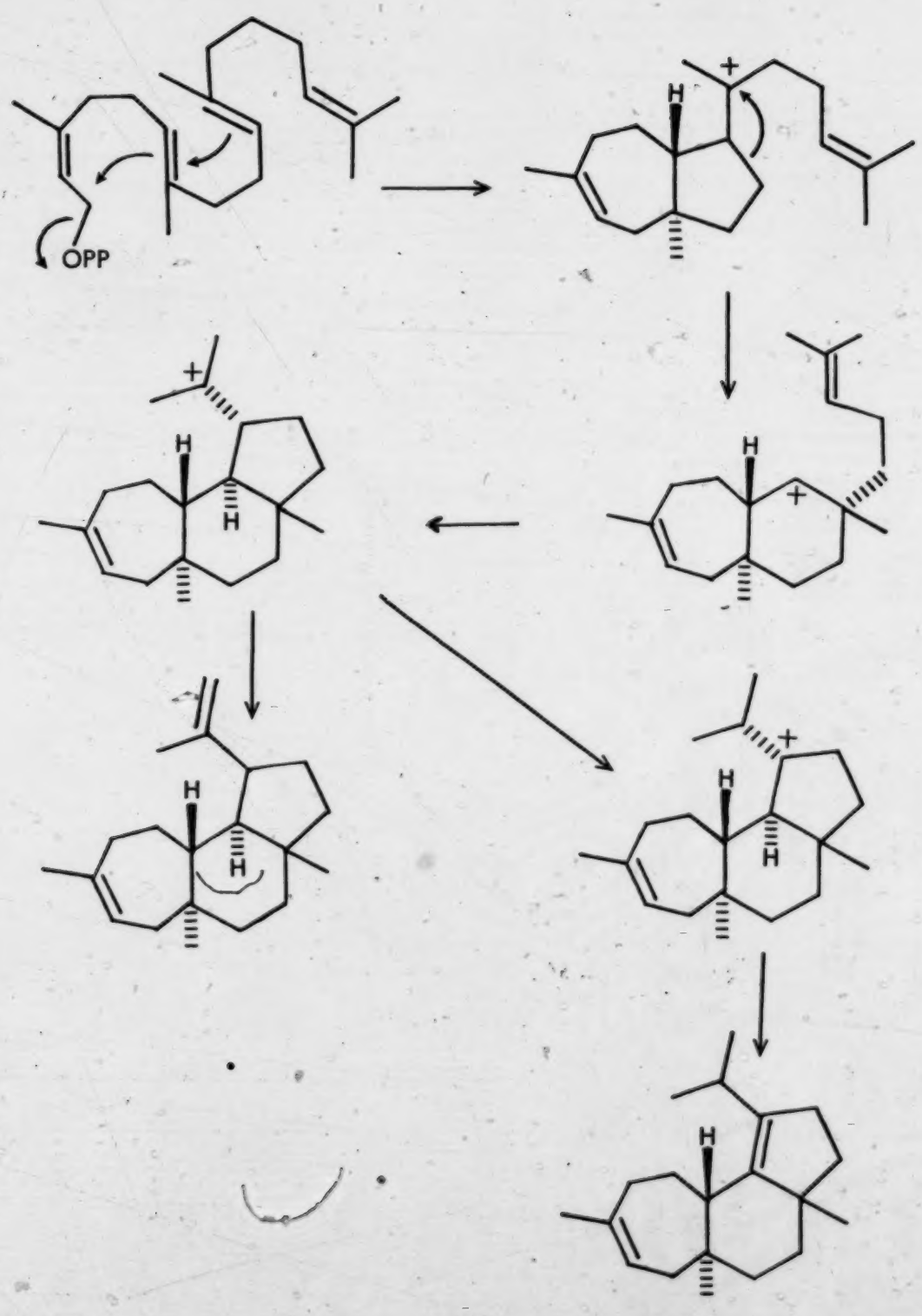


(20)

Although there has been to date no successful work done on the biosynthesis of the cyathins a biogenetic scheme has been advanced.[15] This scheme bears only a little resemblance to the biosynthetic pathway of Iridoids.

In particular the five-membered ring is formed by carbonium ion attack on the isopropylidene residue, whereas in iridoids it seems clear that it is formed by nucleophillic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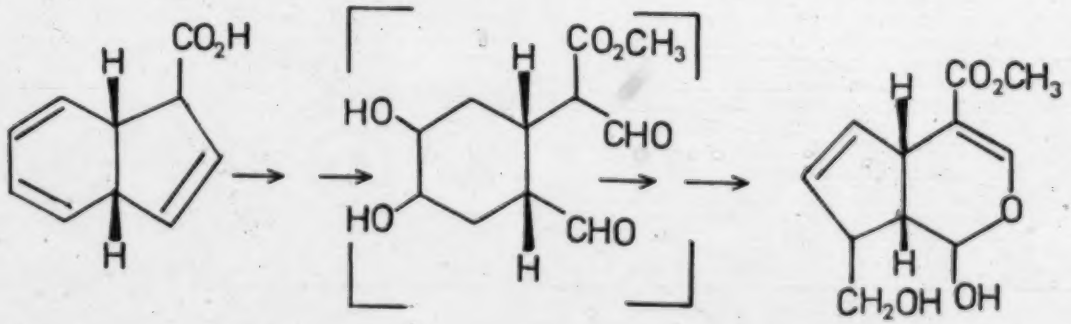
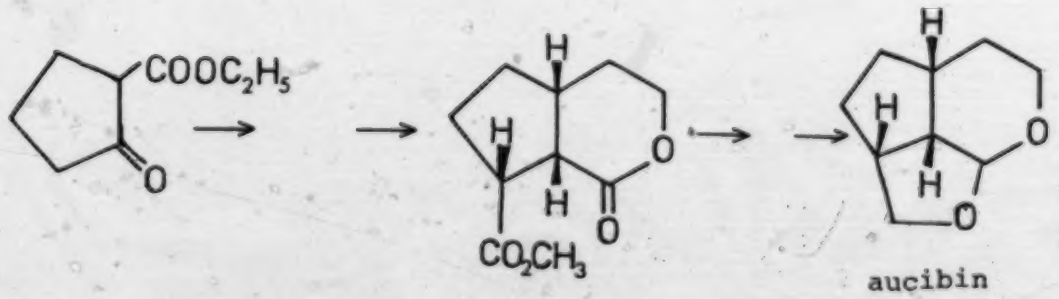
attack on the isopropylidene group.

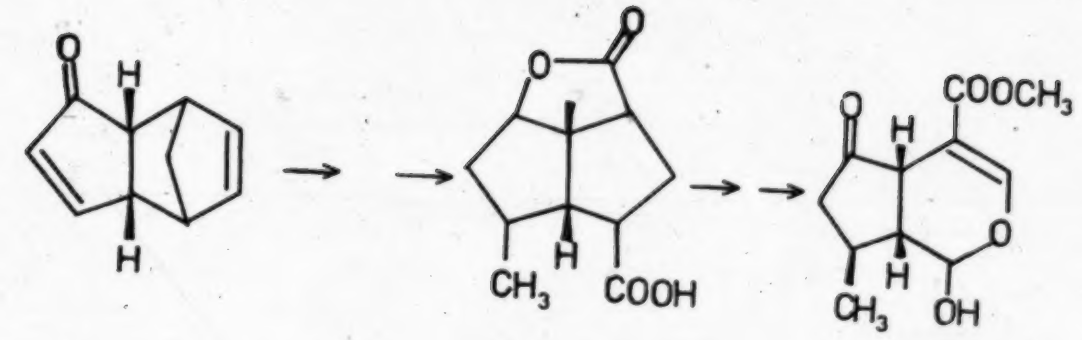
However this is of no consequence when one considers the synthetic approaches to the cyathin structure. First of all the regular synthetic routes to the Iridoids are of little value as most of these start with a pre-formed five-membered ring. The task then becomes simple, as the six-membered ring is easily constructed around this. Apart from Robinson's biogenetic - like synthesis there are no routes to the Iridoids which involve as their final step the formation of a five-membered ring.[16] From the point of view of a cyathin synthesis this is however a critical step. Thus our objective was not simply to investigate a new route to the Iridoids, but also to develop a viable method of forming five-membered rings.



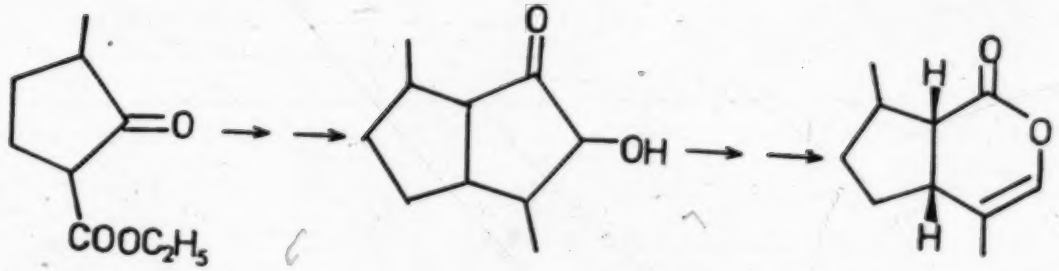
genipin



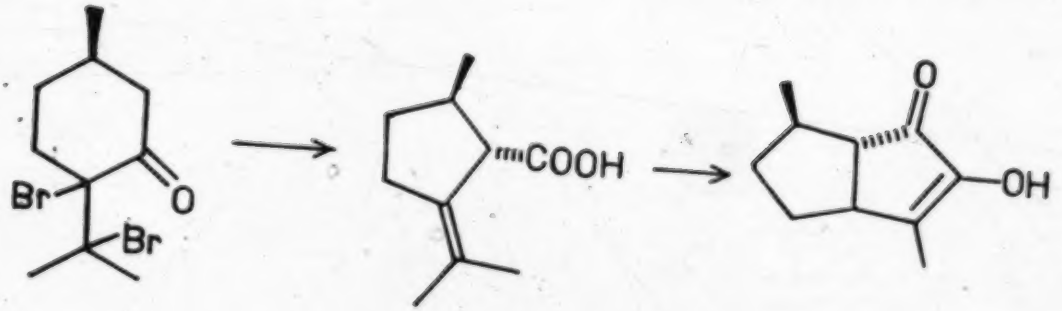
aucibin

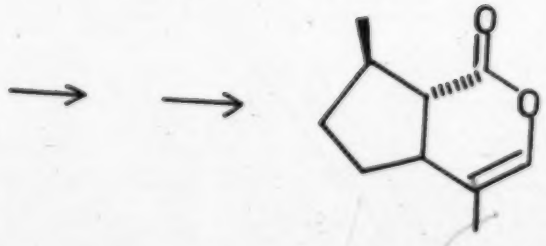


verbenalol



neptalactone

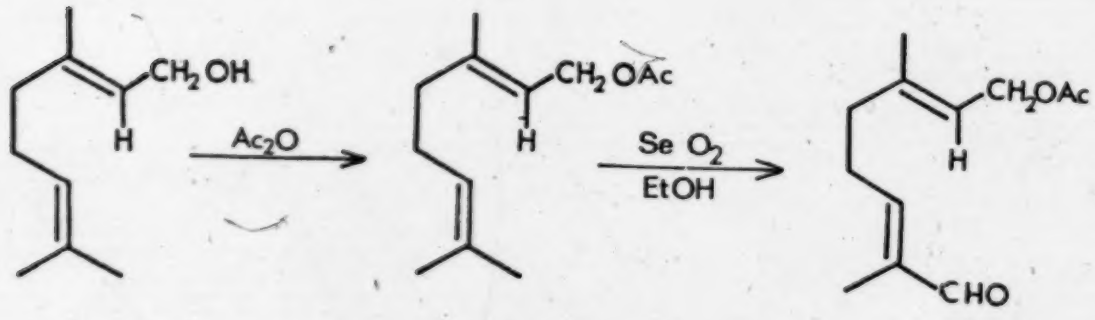


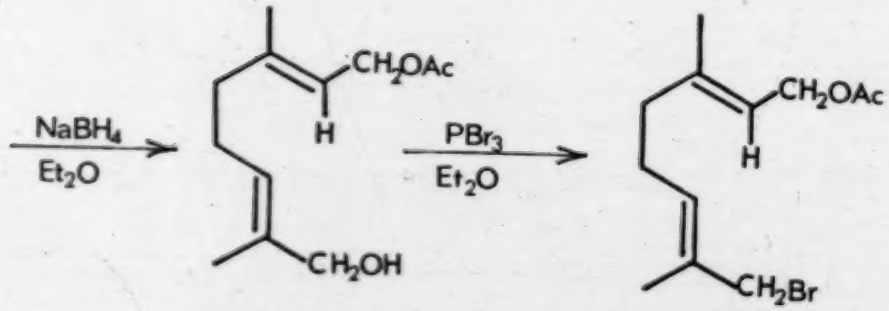


nepetalactone

DISCUSSION AND RESULTS

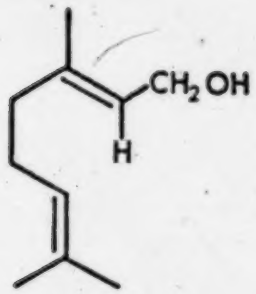
The route to 10-bromogeraniol has already been mapped out in a communication by Grieco.[17] It was decided to follow his scheme in outline. The basic details are shown below.





The acquisition of pure geraniol as starting material presented difficulties. Most commercially available geraniol is synthetic and is at best 50% pure. It is

usually a mixture of the two double bond isomers (21) and (22).



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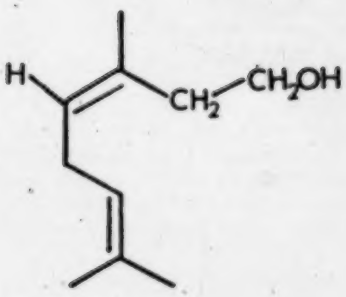


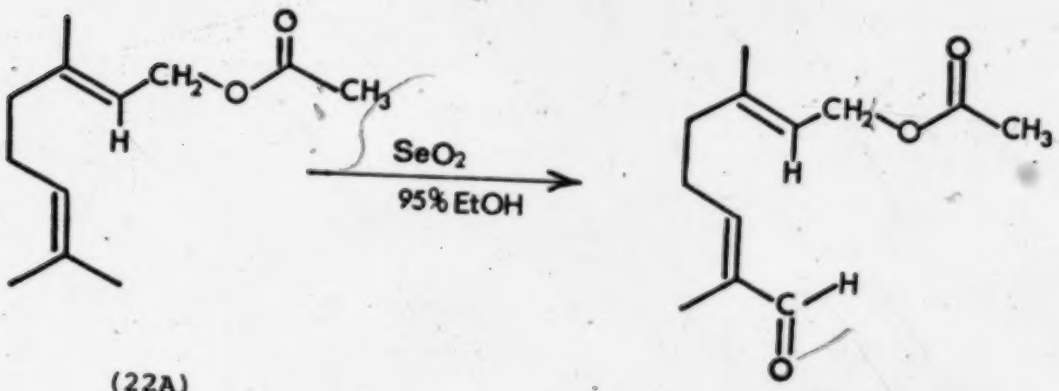
(22)

They are distinguished by the position of the hydroxymethylene absorption in the P.M.R. of these compounds. In geraniol (21) this group appears at δ 4.1 and in the isomer (22) it occurs at δ 3.6.

In separating these compounds we made use of an observation of Jacobsen that geraniol forms a crystalline complex with anhydrous calcium chloride.[18] We found this procedure excellent in every detail. Calcium chloride is added to a solution of geraniol in hexane and cooled to -40°C for 30 minutes. The calcium chloride complex is filtered off and hydrolysed with water. Very pure geraniol is recovered by extracting the aqueous mixture with ether.

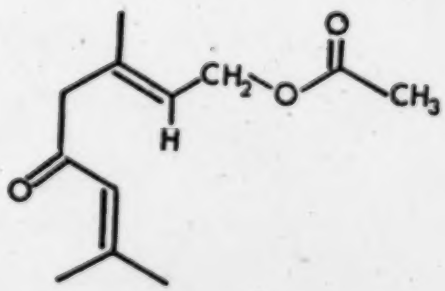
Geraniol was acetylated using acetic anyhdride with sodium acetate as catalyst in benzene.[19] The yield after purification by spinning band distillation was 90%.

The introduction of a functional group at the 10 position of geraniol is an easy matter. It has been known for many years that the reaction of selenium dioxide with geraniol acetate in 95% ethanol produces an aldehyde exclusively at the 10 position.[20]



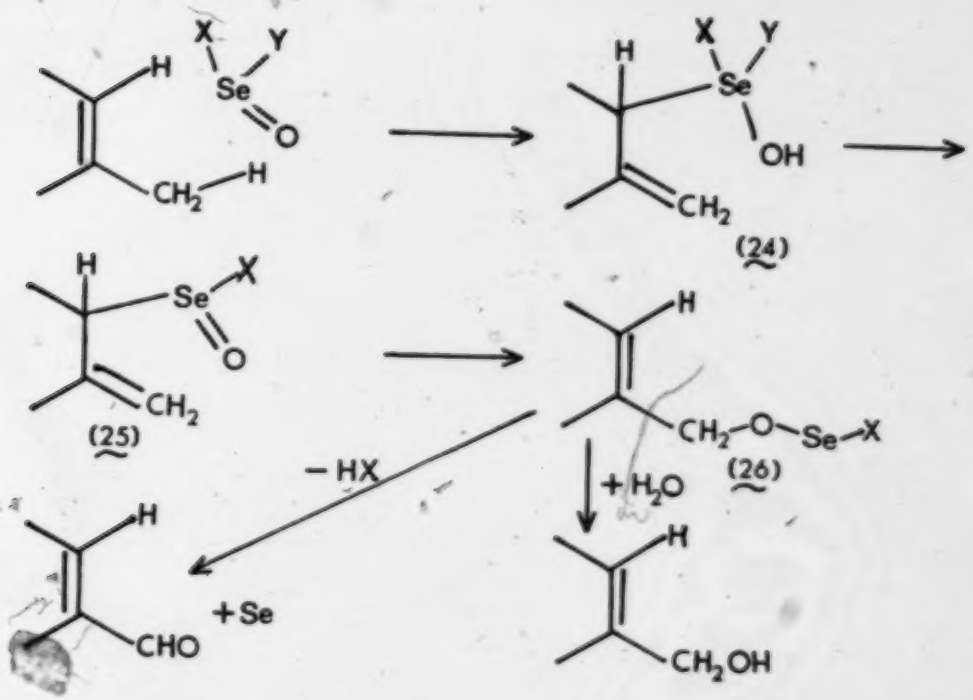
(22A) (27)

This result is in apparent contradiction to the rules developed by Guillemonat for SeO_2 oxidations.[21] According to Guillemonat the main product should be the ketone (23).



(23)

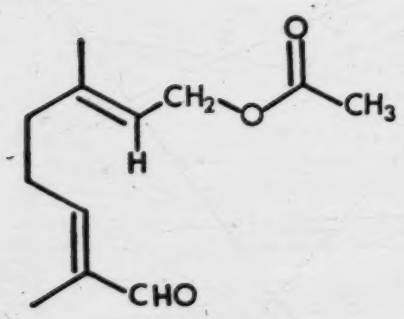
A number of different explanations have appeared to account for this anomaly and also the remarkable stereoselectivity, in that only C-10 aldehyde is produced and not the C-9.[22] However the unravelling of the mechanism of the SeO_2 oxidation of olefins by Sharpless in 1972 offers a clear explanation.[23]



The solvated SeO_2 participates in an ene reaction with the E side of the double bond to give (24) which collapses to the allyl selenic acid (25). This rearranges

by a 2, 3 sigmatropic rearrangement to give the selenium (II) ester (26). This ester is either hydrolysed to the alcohol or eliminates HX and selenium metal to give the aldehyde.

Initial attempts to carry out this reaction using one equivalent of geranyl acetate and one equivalent of selenium dioxide in refluxing 95% ethanol for 1 hour led to a poor yield. The product consisted of 60% starting material and 35% of the alcohol (28). Only a small amount of aldehyde (27) was produced.



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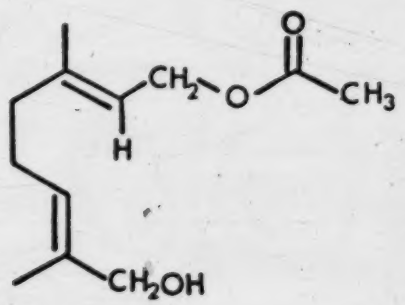
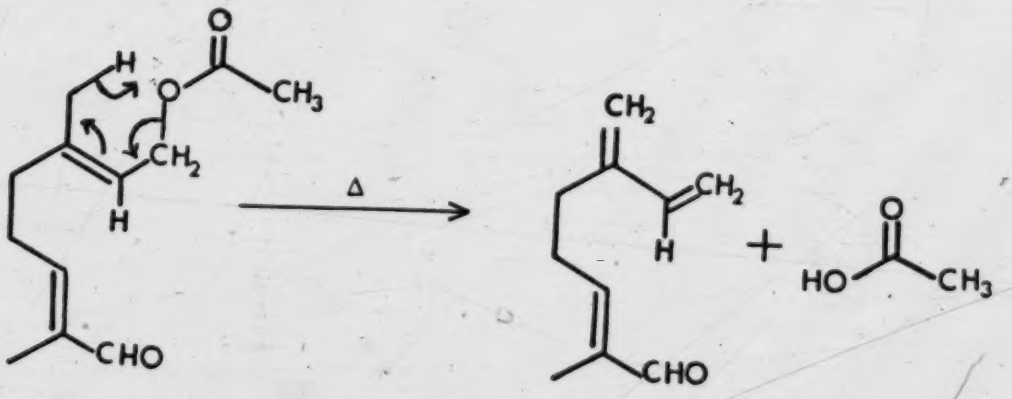


(28)

By doubling the molar ratio of SeO_2/geranyl acetate the yield was markedly improved. The product now contained 11% starting material, 51% aldehyde (27) and 38% alcohol (28). A certain amount of polymeric material was also produced. It was found that by employing inverse addition - that is slowly adding a solution of SeO_2 in ethanol to a refluxing solution of geranyl acetate, the amount of

polymeric material was markedly reduced. There was a considerable improvement in overall yield. There was no change in the ratio of the products.

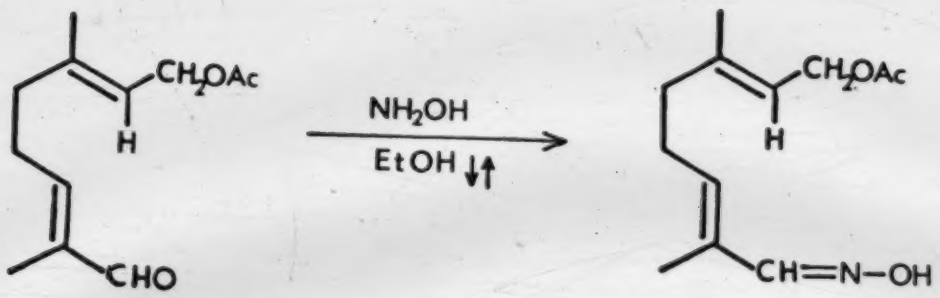
The purification of the aldehyde (27) presented many difficulties. The product could not be distilled, as the high temperatures required caused considerable decomposition. It has been suggested[24] that this is due to the ready elimination of acetic acid *via* a cyclic mechanism.



Column chromatography over silica gel also caused considerable destruction of the aldehyde. Although the gross yield of the reaction was 51% aldehyde, as determined by gas liquid chromatography, the net yield after column chromatography was only 13%.

A number of chemical approaches to the purification were investigated. The aldehyde (27) does not form a bisulphite addition complex. It was reacted with

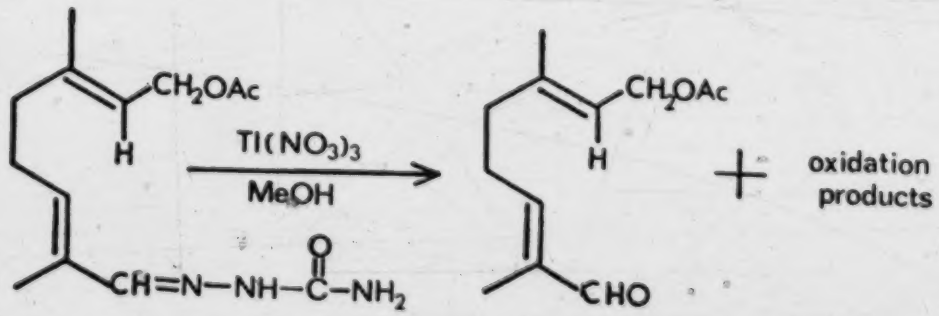
N-hydroxylamine in good yield to produce the oxime (29) but the latter was an oil and could not be crystallized.



(27) (29)

The semicarbazide (30) is readily formed in good yield and is a beautifully crystalline compound.

The semicarbazide was treated with Thallium (III) nitrate according to Taylor and McKillop.[25] This reagent was successful in removing the semicarbazide group but also caused concomitant oxidation at the 2,3 double bond.[26]

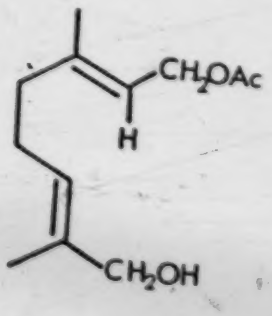


(30) (31)

Molecular distillation under very high vacuum (< 5 microns) was then attempted and this was found to produce reasonable results. In particular geranyl acetate could be separated from the oxidation products and this was the important consideration. The geranyl acetate distilled out of the product mixture at room temperature. By then heating the pot to 70°C and using ethanol at -20°C as the cooling liquid for the condenser - the aldehyde and alcohol distilled over together in good yield and excellent purity.

In later experiments the crude product mixture from the SeO_2 oxidation was used directly in the next step, as it was found that the alcohol (28) could be purified with ease as its complex with calcium chloride.

The reduction of the aldehyde (27) to the alcohol (28) was attempted using $NaBH_4$ in 2-propanol. The product was a mixture of the allylic alcohol (28) and the dihydro alcohol (32). These were separated by G.L.C. and identified by G.L.C.-Mass Spectroscopy.



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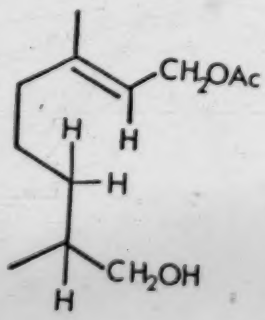


(32)

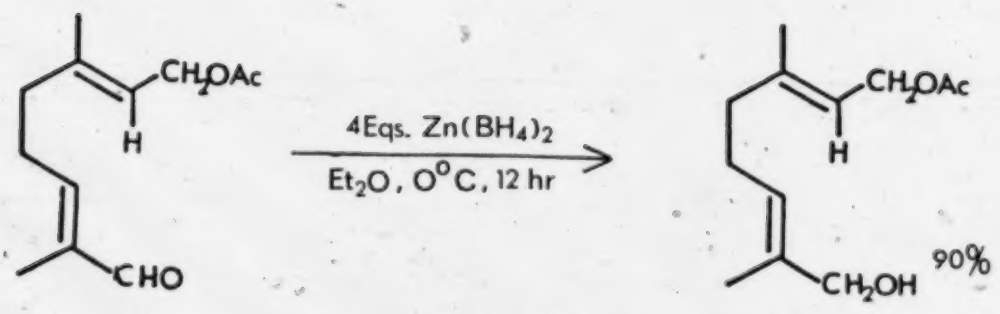
The P.M.R. spectrum of the product indicated a mixture of 65% (28) and 35% (32). The dihydro alcohol (32) showed a methyl doublet at δ 0.9 and a hydroxy methylene doublet at δ 3.45. The desired allylic alcohol (28) showed a pair of vinylic methyl groups at δ 1.7 and a hydroxy methylene singlet at δ 4.0.

The solvent used for the $NaBH_4$ reduction was changed from 2-propanol to diglyme. The results were the same, with the dihydro alcohol and allylic alcohol being produced in similar proportions. Lowering the temperature of the reaction to 0°C also had no effect except to slow down the reaction.

At this point we were mindful of the fact that AlH_3 has been used as the reagent of choice to reduce αβ unsaturated carbonyl systems to allylic alcohols.[27] Although such a reagent could not be used in this case because it would also reduce the acetate function, we took note that it has a considerably more covalent nature than $LiAlH_4$, which it generally replaces in these cases. Thus we were encouraged to look for a more covalent borohydride reagent.[28] Zinc borohydride is such a reagent and it is easily prepared from $ZnCl_2$ and $NaBH_4$.

Initial experiments using $Zn(BH_4)_2$ in twofold excess led to incomplete reduction over a 12 hour reaction time at 0°C in ether. Simply doubling the quantity of reducing agent led to complete reduction under similar conditions.

The yield of the desired allylic alcohol (28) was 90%.



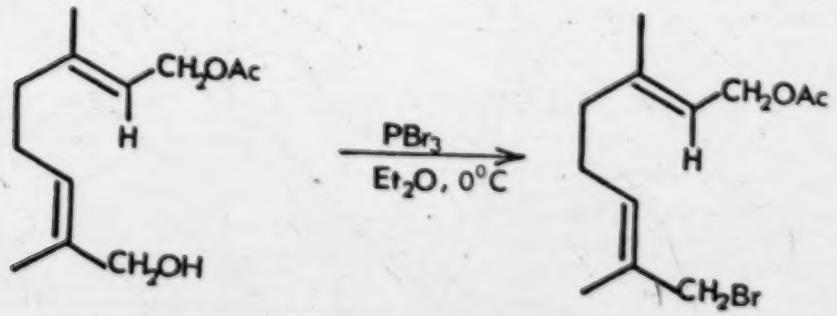
(27) (28)

This alcohol (28) could be purified by a number of techniques. Initially it was chromatographed on dry column silica gel (Woelm Grade No. 2). However as it is colourless and lacks appreciable U.V. absorption at 254 nm it was difficult to locate on the developed column. As it is an allylic alcohol and similar in many respects to geraniol, the use of anhydrous calcium chloride was investigated. The results of this experiment were variable. On some occasions when calcium chloride was added to a hexane solution of the allylic alcohol (28), an immediate reaction occurred, and a conglomerate precipitated. This conglomerate could be formed into a ball which was easily removed as one piece and then hydrolysed in diluted $NaHCO_3$ solution. This method produced very pure allylic alcohol (28).

However on occasion the calcium chloride failed to react with the alcohol. Whether this was due to variation in the quality of the calcium chloride is difficult to say, it may instead have been due to trace amounts of moisture or other impurities in the alcohol. By switching to magnesium chloride instead of calcium chloride uniformly good results were obtained.[29] The magnesium chloride was easily prepared by heating a mixture of magnesium chloride hexahydrate and thionyl chloride. Evaporation of the volatile components followed by heating to 90°C under high vacuum gave pure anhydrous magnesium chloride.

The alcohol (28) could be oxidized to the starting aldehyde (27) using activated MnO_2 in methylene chloride.[31] The reaction is slow but proceeds to completion in 48 hours. This confirms that the reduction proceeded without isomerization about the 6-7 double bond.

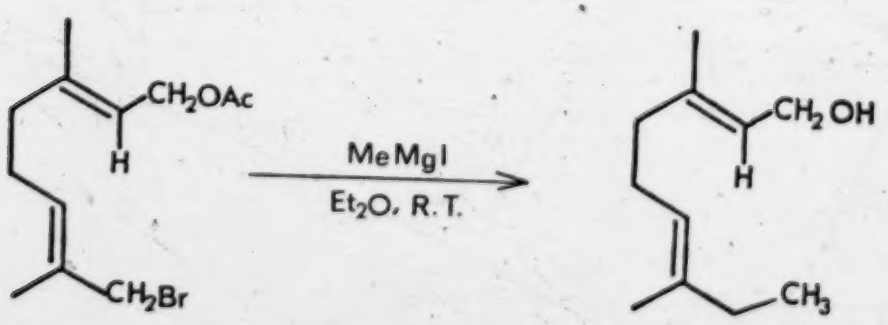
The allylic alcohol was converted into the allylic bromide (33) by treatment with phosphorous tribromide in ether at 0°C for 12 hours.[17]



(28) (33)

The yield is 46%. This reaction proceeds slowly and the yield is markedly reduced by shortening the reaction time. It was found that the yield could be improved and the reaction time shortened by adding lithium bromide to the reaction. This speeded up the reaction by increasing the concentration of bromide ion.

The conversion of the bromoacetate (33) into the bromoalcohol (34) was not straight-forward. Methyl magnesium iodide was first investigated, since this should produce the iodomagnesium salt of the alcohol. Reaction of 2.2 equivalents of methylmagnesium iodide with 1 equivalent of bromoacetate (33) led to a mixture of two products. Analysis by T.L.C. indicated a new compound more polar than the starting material and therefore corresponding to an alcohol. The component matched the r.f. of geraniol (21).



(33) (35)

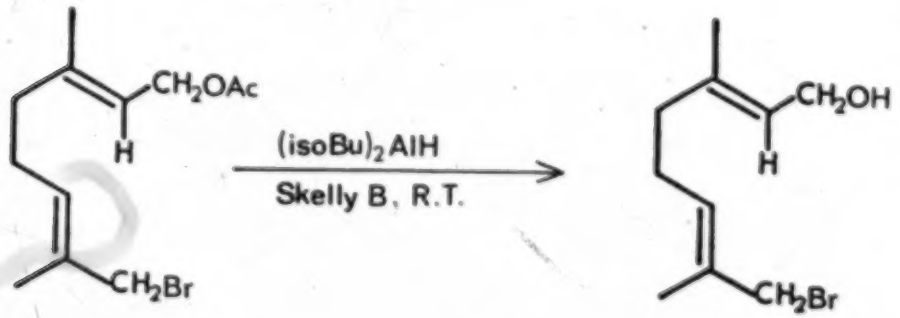
The more polar component of the mixture was isolated by prep dry column chromatography on silica gel. It was readily identified by P.M.R. spectroscopy as 10-homo geraniol (35). The new methyl group appears as a triplet at δ 0.95 and the C-10 methylene as a quartet at δ 1.90. The I.R. spectrum lacks a carbonyl function but contains a hydroxy group.

This compound (35) was best prepared by adding 3.5 equivalents of methyl magnesium iodide to 1 equivalent of the bromoacetate (33) in which case it became the only product. The same result was achieved using methyl-magnesium bromide. The coupling of allylic halides with Grignard reagents is well documented.[55]

The cleavage of acetate groups is well documented in the chemical literature and there are abundant methods for performing this task.

These methods consist of two types: those involving hydrolysis (or alcoholysis) in basic media, and those involving reduction, usually by metal hydrides. Of the former, the use of 10% aqueous Na_2CO_3 and 10% aqueous $NaHCO_3$ was found to leave the acetate group intact. Methanol with a catalytic amount of sodium methoxide was also without effect. In this case however it is reasonable to assume that the catalyst was consumed by reaction with the allylic bromide.

The use of metal hydride reducing agents was then considered. The reaction of lithium aluminium hydride with the bromoacetate (33) in ether gave rise to a mixture of products. This same result was obtained despite variation in temperature and modifications such as inverse addition. Di-isobutylaluminum hydride was then considered and found to react with the bromoacetate (33) in Skelly B. to give, as the only product the desired bromoalcohol (34).



(33) (34)

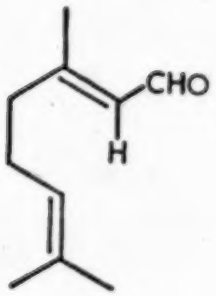
The P.M.R. spectrum of (34) shows the hydroxymethylene at δ 4.0 as a doublet. It exhibits an upfield shift of 0.5 PPM compared to (33) which is characteristic of the conversion of a primary acetate group to alcohol.

The scene was now set for the attempt at forming the five-membered ring using the mixed Grignard reagent/ Grignard salt method. It was anticipated that treatment of (34) with 1 equivalent of methylmagnesium bromide would

quantitatively produce the Grignard salt. Reaction of the Grignard reagent with the allylic bromide would be avoided by the increased reactivity of the allylic alcohol towards such a reagent.

Therefore a solution of (34) in tetrahydrofuran was treated with 1 equivalent of methylmagnesium bromide at 0°C under N_2. After 10 minutes finely divided magnesium powder was added to the reaction flask and the mixture stirred at room temperature for 12 hours. Upon work-up, the product of the reaction was found to be a mixture of many compounds. The exact number of these was undeterminable by T.L.C. on silica gel. However the mixture had a strong odor of citral and one of the spots on T.L.C. corresponded in R.f. value to that of citral. This spot also quenched fluorescent indicator at 254 nm and had a slight yellow appearance. It was isolated by prep dry column chromatrography on silica gel (Woelm Grade No. 2) and identified as citral by its infrared spectrum. Because citral itself is a mixture of geranial and neral it was of interest to determine by P.M.R. spectroscopy whether the synthetic product was also a mixture. Commercial citral shows two aldehyde protons at δ 9.85 and δ 9.93. These correspond to neral and geranial respectively. The synthetic sample showed only one aldehyde proton at δ 9.91 thus indicating that it has geranial stereochemistry.[32] The presence of multiplets at δ 5.0 and δ 4.65 indicate

that it is a mixture of the double bond isomers (36) and (37).



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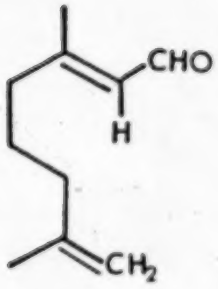
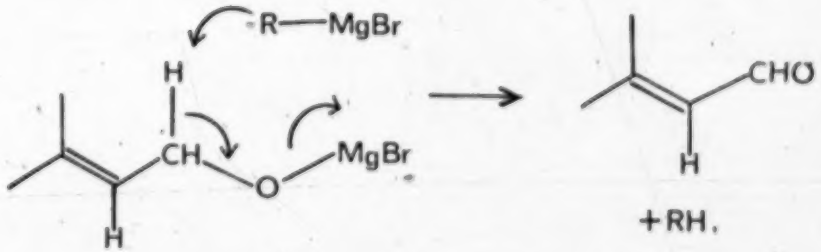
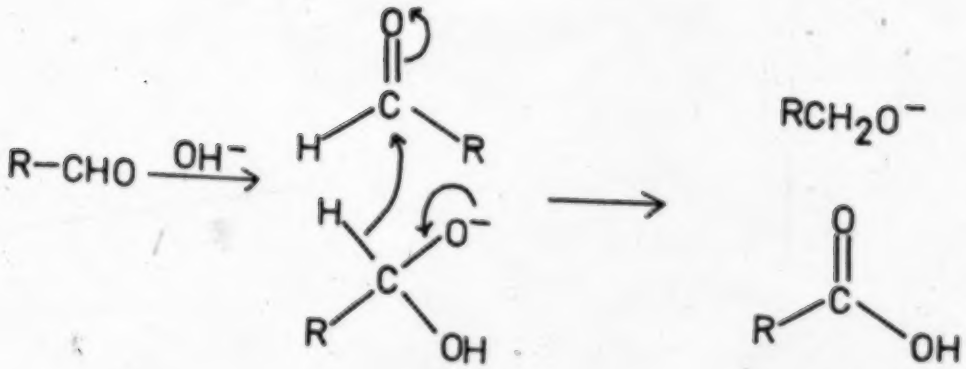


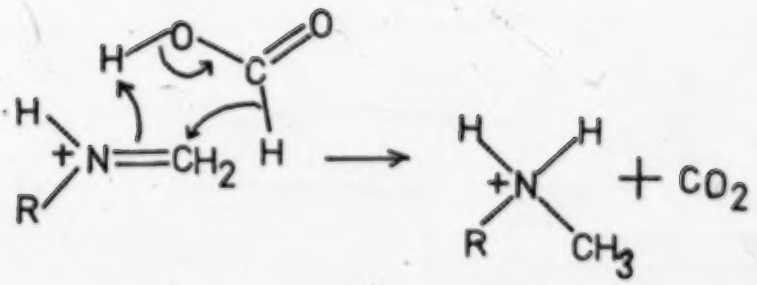
(37)

This result is tentatively explained as oxidation of the Grignard salt by the Grignard reagent, e.g.,

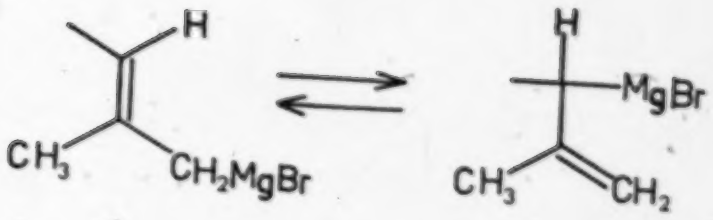


The product is therefore the aldehyde of the Grignard salt and the hydrocarbon of the Grignard reagent. A similar mechanism has been proposed for the Cannizzaro and Eschweiler-Clark reactions, e.g.,





The isomerization of the allylic Grignard reagent is a well known phenomenon and the equilibrium between the two forms has been shown to be very fast.[33]



The failure of the desired cyclization to take place was a great disappointment. The reason for this failure was, however, far from clear. That the Grignard reagent had indeed formed there can be little doubt. The isomerization of the isopropylidene double bond was clear evidence of this. This Grignard reagent would never have formed if the allylic alcohol had not already been converted to the Grignard salt. Thus it can be assumed that the desired starting material for the reaction had been produced.

There are two possible reasons for the reaction to fail in this particular starting molecule. The configuration of the reactant could be such that the two reactive sites could not come together. Alternatively the electrophillicity of the Grignard salt could have been insufficient. The latter seemed reasonable, as it has been reported that

nucleophilic addition to double bonds is strongly dependent on substitution - the more substituted double bonds reacting slowest.[34] In the case in hand the double bond is trisubstituted and some difficulty can be expected.

The electrophilicity of the double bond could be easily tested by reaction with the most active of Grignard reagent towards nucleophillic addition - allylmagnesium bromide.[35]

A solution of allyl magnesium bromide in di-n-butyl ether was prepared according to Turk.[36] The solution was very cloudy due to suspended $MgBr_2$. This was remedied by filtration under a dry N_2 atmosphere. It was analysed by acidimetric titration and found to be 0.24 M. Although di-n-butyl ether is not the reagent of choice for preparing allylic Grignard reagents, as it promotes coupling more than any other ether,[35] in this case coupling is not so much a problem as it is with other substituted allylic halides. It also has a high boiling point and it was felt it might be important to investigate the effects of heat on the addition reaction.

Accordingly, 1 equivalent of geraniol was reacted with 2.3 equivalents of allyl magnesium bromide in di-n-butyl ether under nitrogen. At room temperature over a long period of time, no addition to the double bond took place. This was monitored by withdrawing aliquots of the reaction mixture and analysing them by G.L.C. on F.F.A.P.

Refluxing of the reaction solution resulted in the production of a single new compound in about 25% yield. It was isolated and purified by prep dry column chromatography on silica gel (Woelm Grade No. 2) followed by molecular distillation at 10 microns. The I.R. spectrum showed only hydrocarbon absorptions. The presence of a double bond(s) was indicated by bands at 1645 and 3070 cms^{-1}.

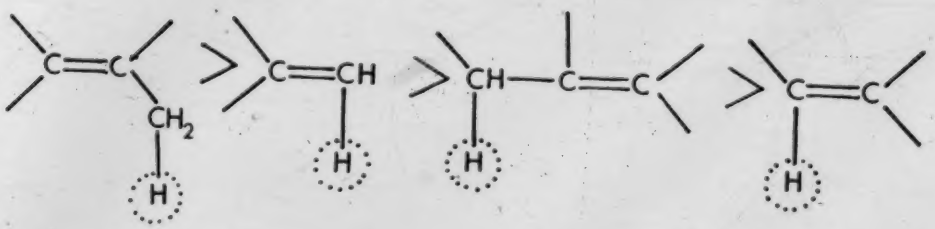
This was confirmed by the P.M.R. spectrum which showed only olefinic, vinyl and allylic absorptions. The mass spectrum had a parent peak at m/e 274 which was $C_{20}H_{34}$ (as determined by exact mass measurement). Thus the compound is a coupling product of geraniol formed by the forcing condition of the reaction. It was not investigated any further.

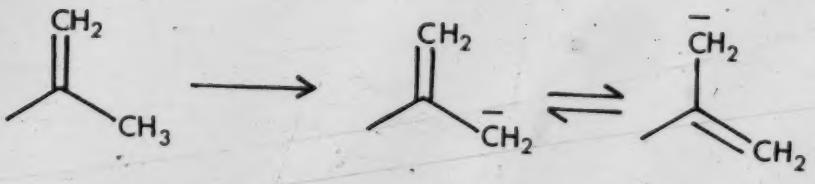
The failure of allylmagnesium bromide to add to the 2,3 double bond of the Grignard salt of geraniol raises serious doubts concerning the feasability of the proposed synthetic scheme. The electrophilicity of this double bond is of crucial importance. It was decided to test the reactivity of this double bond in another series of reactions.

The addition of alkyl lithium reagents to lithium salts of allyl alcohols is a well known reaction.[35,37] It even proceeds in certain cases where Grignard reagents fail - i.e., the addition of alkyl groups rather than allyl groups to double bond. The addition proceeds with difficulty, however, in the case of γ substituted double bonds, which

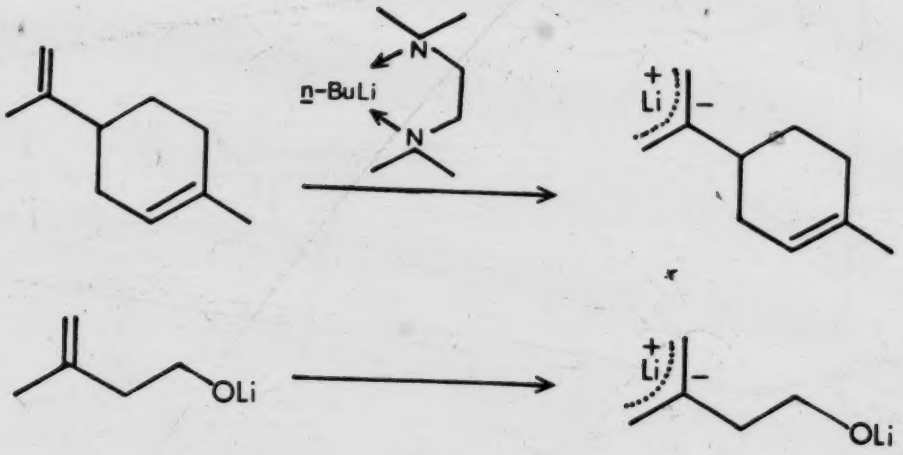
is of course the case with 2,3 double bonds of geraniol. It was felt however that little could be lost in testing the possibility because in doing so a second synthetic possibility could also be investigated.

It was shown by Morton that olefinic hydrogens could be metalated under certain conditions.[38] In a revision of his work Broaddus developed the following order for the ease of metalation.[39]

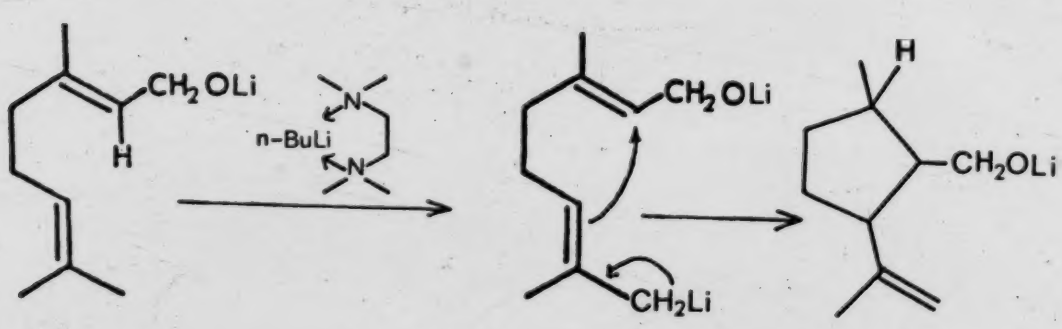




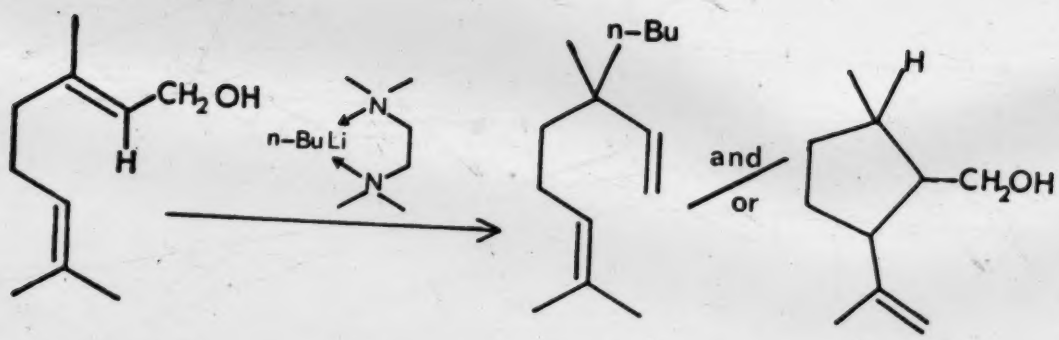
This is aptly demonstrated by the ease of metalation of limonene[40] and 3-methyl-3-buten-1-ol.[41]



The isopropylidene terminus of geraniol contains two vinyl methyl groups corresponding to category one. If these were sufficiently acidic they might generate the alkyl lithium reagent necessary for an intramolecular addition to the allylic alcohol, e.g.



Thus there are two synthetic possibilities for the reaction of ^{n}BuLi/TMEDA with geraniol



The opportunity for killing two birds with one stone having thus presented itself, it was with great anticipation that the reaction was investigated.

As the addition of alkyl lithiums to allylic alcohols does not require the presence of tetramethylethylene diamine (TMEDA) the reaction was first attempted omitting this. This should distinguish between the two possible reaction modes.

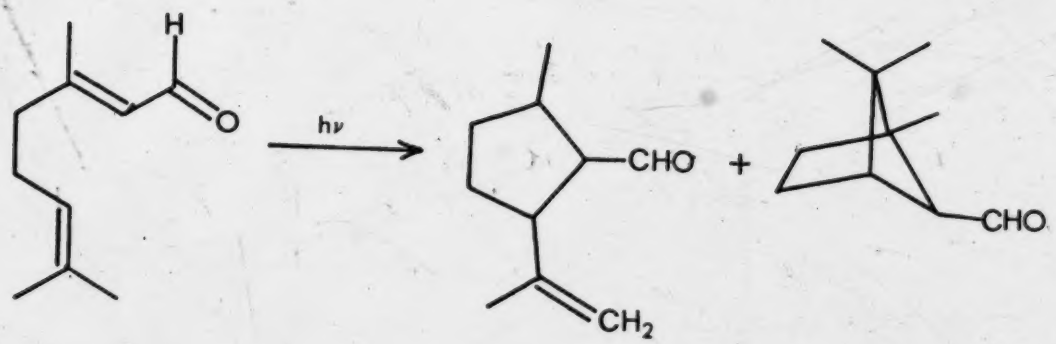
Between geraniol and 3 equivalents of ^{n}BuLi in hexane at room temperature over long periods of time there was no discernable reaction other than formation of the lithium alkoxide. The implication of this was clear - there was no addition across the allylic alcohol double bond of geraniol.

Repeating the experiment with 1 equivalent of geraniol, 1 equivalent of TMEDA and 3 equivalents of ^{n}BuLi, gave at

room temperature after 24 hours, a single new product which was 25% of the product mixture, the rest being unreacted geraniol. This was isolated by prep T.L.C. on silica gel. From its behaviour on T.L.C. and the presence of only C-H bands in the I.R. this compound was obviously a hydrocarbon. The P.M.R. was complex and offered little information. The mass spectrum indicated a mixture of isomers of the general formula $C_{24}H_{42}$. This implies the addition of one butyl group and the dimerization of two geraniol units. The compound was not investigated further as the reaction appeared to have no synthetic value.

The obvious unreactivity of the 2,3 double bond of geraniol or its derivative toward nucleophilic addition, forced a reappraisal of the synthetic approach. Any method based on such a reaction seemed doomed to failure. We were therefore obliged to consider the alternative methods of joining carbons 2 and 7 of geraniol. In a general way there were two approaches still to be tried. These were photochemical and electrophilic addition. Because of the vast amount of literature data and certain close analogies we decided to try the photochemical method first.

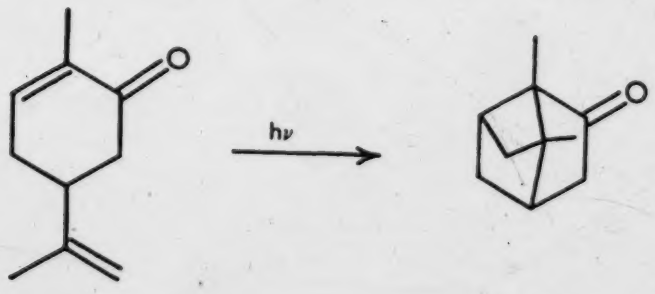
In 1963 Cookson reported that citral when irradiated with medium pressure mercury ultra violet light gave rise to two compounds photocitral (A) (38) and photocitral (B) (39).[43]



(38) (39)

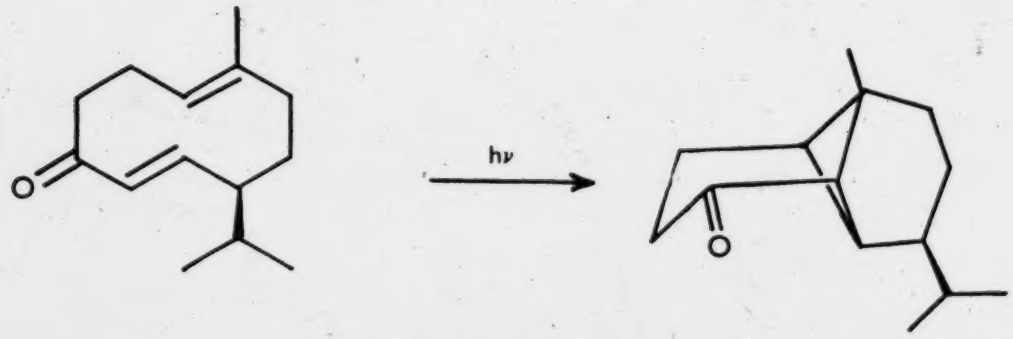
The structure of photocitral (A) is particularly interesting as it has an iridoid structure. Unfortunately Büchi later showed that the stereochemistry was incorrect, in that the methyl and formyl groups were cis rather than trans.[44]

A similar type of reaction had been reported earlier by Büchi for carvone (40), the product being carvone camphor (41).[45]



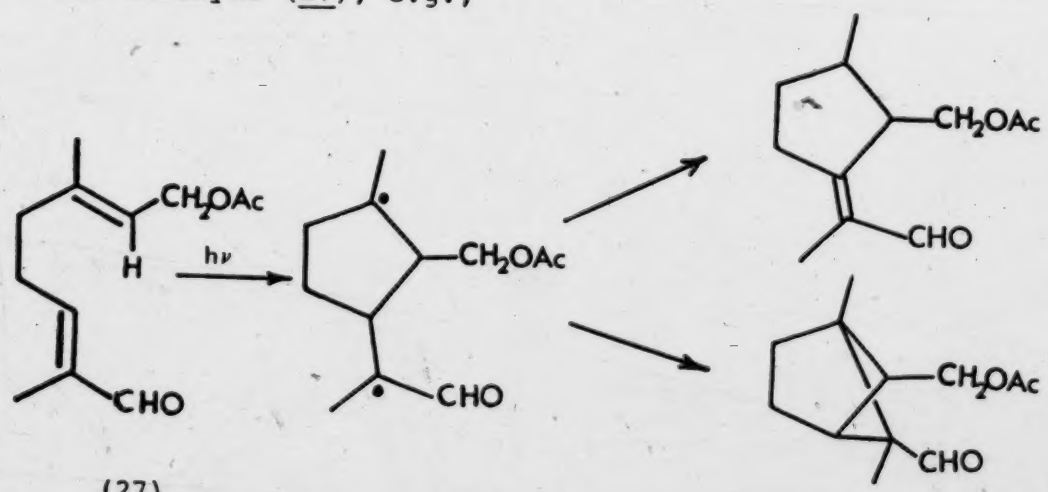
(40) (41)

Heathcock photolysed the cyclodecadienone (42) to give the ketone (43) in substantial amounts.[46]



(42) (43)

The ease and generality of these examples encouraged our belief that a similar reaction would occur in the case of the aldehyde (27), e.g.,



(27)

Accordingly the aldehyde (27) was dissolved in hexane and irradiated with a medium pressure mercury ultra violet lamp. A pyrex filter was used to remove radiation below

320 nm. After one week there was no detectable new compound in the solution.

A report by Hart indicated that intramolecular photocyclizations of this type could be speeded up by supporting the starting molecule on silica gel suspended in cyclohexane or by performing the reaction in trifluoroethanol.[47]

The first of these modifications was attempted with no success. After irradiation for one week in cyclohexane/silica gel with a medium pressure U.V. mercury lamp, the aldehyde (27) was recovered unchanged. Analysis by G.L.C. of the reaction medium also indicated no change had occurred.

The solvent was changed to trifluoroethanol and the previous reaction conditions were repeated. On this occasion although no new products could be detected by G.L.C. a definite change occurred in that the geranyl acetate (which was present as an impurity in the aldehyde) gradually disappeared over the course of a week. The aldehyde itself remained unchanged.

This observation, which was somewhat unexpected, was corroborated by repeating the reaction with pure geranyl acetate. As the aldehyde was obviously acting as a photosensitizer for the geranyl acetate, which has no absorption above 320 nm, it was replaced with benzophenone. After irradiation for one week, in trifluoroethanol, under the previous conditions, the geranyl acetate had decreased by

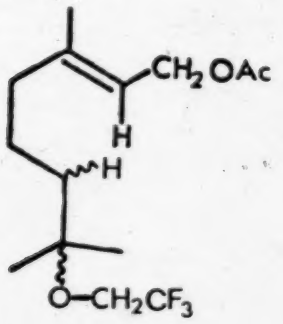
50%. The new product(s) was unstable to G.L.C. analysis, as its presence could only be detected by a broad band of variable shape, indicative of decomposition. It was readily detected by T.L.C. analysis, as a double spot, considerably more polar than geranyl acetate. It was isolated by prep T.L.C. on silica gel and recovered as a light yellow oil.

The infrared spectrum of this compound was very similar to that of geranyl acetate. It showed very clearly an acetate carbonyl at 1740 cms^{-1}, identical with that of geranyl acetate.

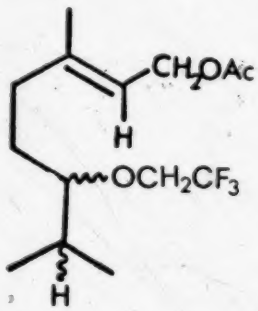
The P.M.R. spectrum, however, showed little similarity to that of geranyl acetate. It contained a considerable aliphatic proton region centered at δ 1.18 equivalent to two methyl groups. A vinylic methyl appeared at δ 1.7 and the acetate methyl at δ 2.0. The acetate methylene appeared at δ 4.50 obviously coupled to an olefinic proton at δ 5.3. Thus carbons 1 to 5 of geranyl acetate are still intact, including the acetate function. The C7-8 double bond is no longer present and the C-9 and -10 methyl groups are aliphatic in nature.

Since the only possibility was the addition of some molecule to the C7-8 double bond, the probable structure now involved a molecule of trifluoroethanol. This was confirmed by the F^{19} M.R. spectrum. It showed two trifluoromethyl groups at δ 7.48 and δ 7.72. Both of these were

triplets and showed a splitting of 8 Hz. The absorption at δ 7.72 was roughly 10% of the δ 7.48 absorption. On the basis of this, two structures were postulated for the addition compounds. These were (42) and (43).



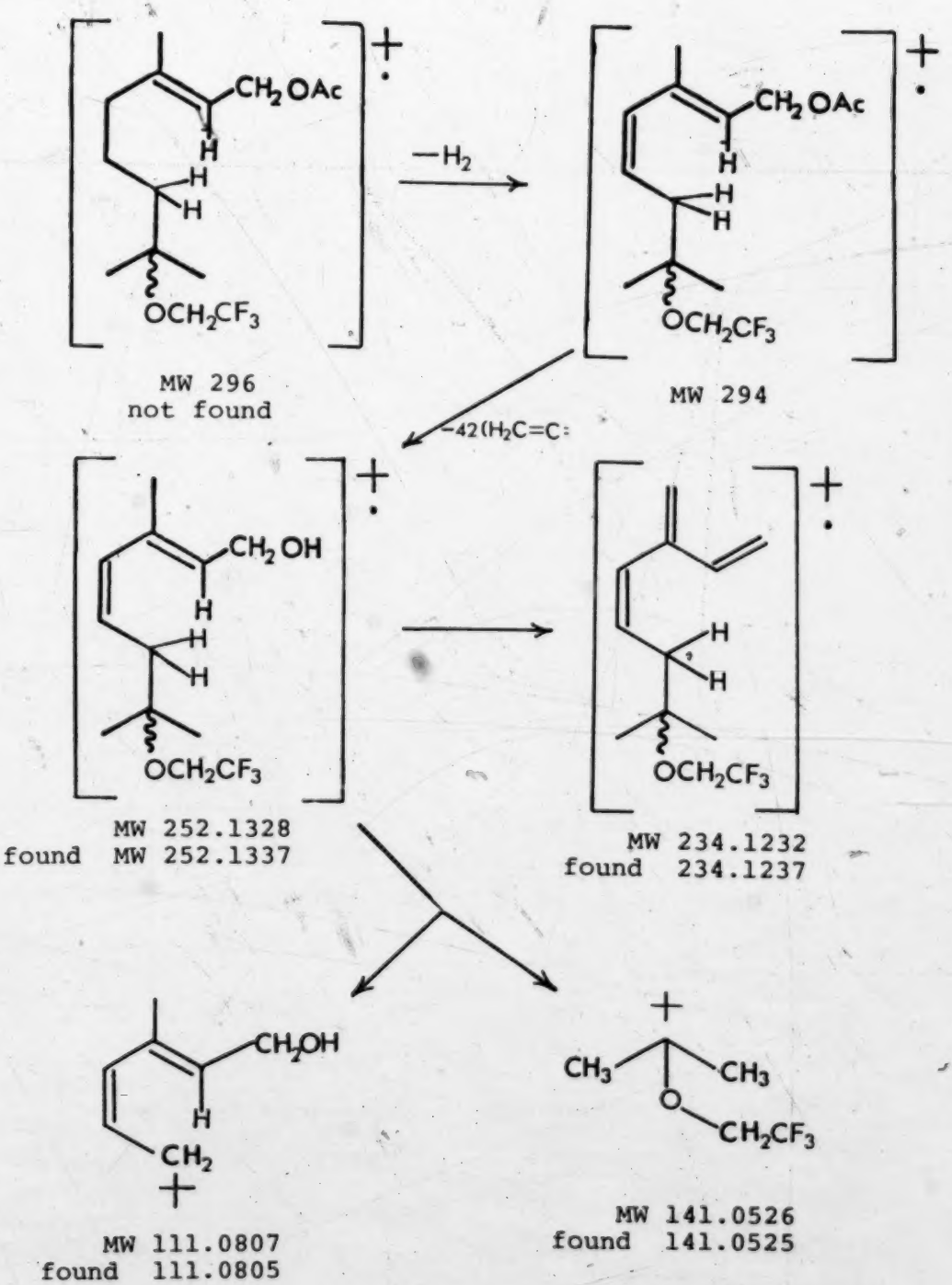
(42)



(43)

It also seemed reasonable that the reaction product was a mixture of these two in the ratio (42)/(43) = 10/1. Structure (42) was chosen as the main product, as the aliphatic methyl groups were clearly singlets in the P.M.R. spectrum.[47a] This also accounts for the double spot that was observed on T.L.C. and the poor stability of these compounds to G.L.C. analysis.

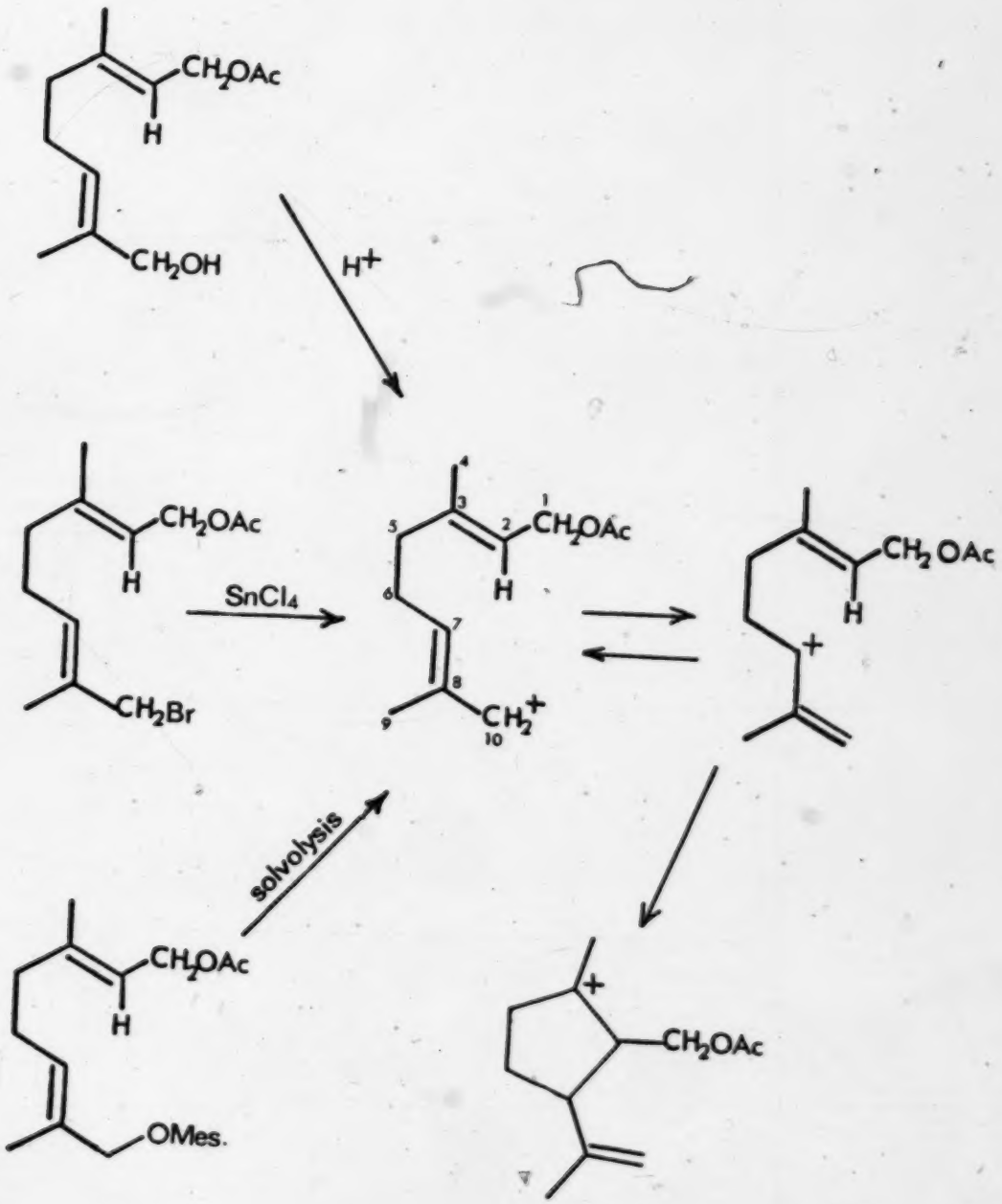
Further proof was given by the mass spectrum of these two compounds. This is outlined in the following scheme:


CH2OAc
OCH2CF3
−H2
MW 296
not found
MW 294
−42(H2C=C:
CH2OH
MW 252.1328
found MW 252.1337
MW 234.1232
found 234.1237
CH2OH
CH2
MW 111.0807
found 111.0805
CH3
CH3
O
CH2CF3
MW 141.0526
found 141.0525

As a result of this the photochemical approach to the iridoids was not investigated further.

Following the failure of the photochemical experiments to lead to any cyclized product, we decided to use the only route, that was as yet untested. This involved electrophilic attack of the carbonium ion on the 2,3 double bond of geraniol.

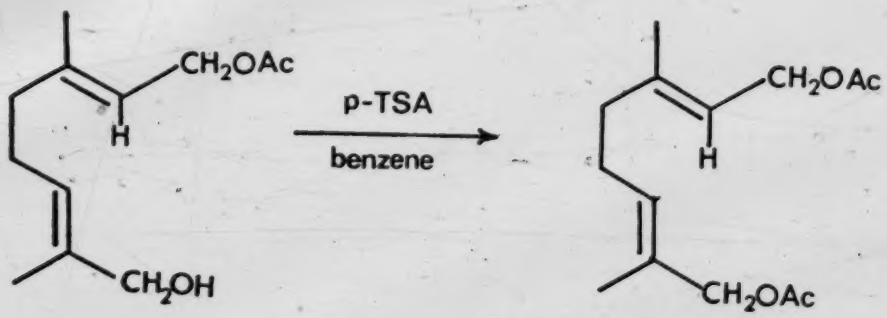
The obvious site for the carbonium ion was at C-7. However, we were well supplied with compounds derivatized at C-10, and it seemed reasonable that a C-10 carbonium ion would be equivalent to one at C-7. In fact a carbonium ion generated at C-10 should exist mainly as a C-7 ion, due to the fact that the latter is disubstituted.


CH2OAc
H
CH2OH
H+
CH2OAc
H
CH2Br
SnCl4
CH2OAc
H
CH2+
CH2OAc
H
+
solvolysis
CH2OAc
H
OMes.
+
CH2OAc

The carbonium ion at C-10 could be generated in a number of different ways. The simplest method involved protonation of the C-10 alcohol.

Alternatively a C-10 bromine group could be removed using stannic chloride. Failing this the C-10 mesylate or tosylate could be pressed into service.

Our initial experiments involved the hydroxy acetate (28). Treatment of (28) with a catalytic amount of p-toluene sulphonic acid in benzene led to the formation of a single, new, less polar compound. This was isolated and identified as the diacetate (34).



(28) (34)

The P.M.R. spectrum of (34) shows two acetate methyls at δ 1.97 and δ 1.98. The C-10 methylene occurs at δ 4.35, exhibiting the characteristic downfield shift observed at the 2 carbon for the conversion of a primary alcohol to an acetate. The mass spectrum was determined using chemical ionization with ammonia. The base peak corresponded to the parent peak

at m/e 272 (M+18). A similar peak occured at m/e 526 (2M+18).

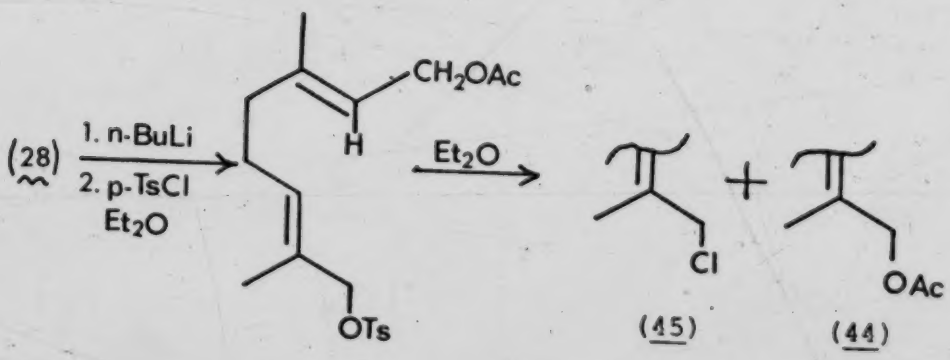
In retrospect this result was not unusual. The p-toluenesulphonic acid catalyses not only the formation of the carbonium ion at C-10 but also the alcoholysis of the ester at C-1. That the latter reaction should be the dominant one indicates both the already well observed lability of the allylic acetate but also the sluggishness of the carbonium ion at C-10 towards rearrangement. That no other products were observed was not unusual - any dienes must certainly have polymerized under the reaction conditions. A yield of less than 50% was observed, in keeping with the mechanism.

Clearly, a new approach to the generation of C-10 carbonium ion was required. It had to be formed under conditions which did not concomitantly affect the allylic acetate. Stannic chloride was decided upon as a reagent for this task. This should complex selectively with the bromine at C-10, and generate the carbonium ion.

Treatment of 10-bromogeranyl acetate (33) with stannic chloride[48] in Skellysolve B at -78°C led to no detectable product. At 0°C the reaction was too vigorous and produced a plethora of products. The P.M.R. also indicated a continuum. It was decided that stannic chloride is too powerful a reagent in this case as the products of the reaction were unstable in its presence.

The simplest and mildest method of generating carbonium ions is by solvolysis of tosylates or mesylates. This method had not been tried earlier as allylic tosylates and mesylates are difficult to form and manipulate. Failing the previous two procedures we resolved to test this approach.

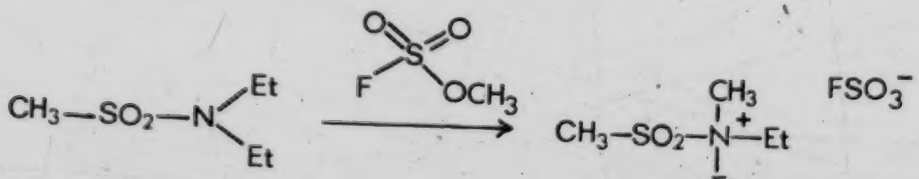
The C-10 tosylate of geranyl acetate was prepared by treating (28) with 1 equivalent of ^{n}butyl lithium in ether, followed by one equivalent of p-toluenesulphonyl chloride.[49] The reaction mixture was stirred overnight and upon work-up contained two new compounds. These were separated by prep dry column chromatography on silica gel (Woelm Grade No. 2) and identified as 10-chloro geranyl acetate (45) and 10-acetoxy geranyl acetate (44).



The P.M.R. spectrum of (45) showed a singlet at δ 4.92 corresponding to the C-10 methylene. Its mass spectrum, as determined by chemical ionization with ammonia, showed

a base peak at m/e 248 (M + 18). The characteristic splitting pattern observed in chlorine compounds was found for the m/e 248/250 peaks and the m/e 206/208 peaks. The latter peaks correspond to the loss of ketene - a reaction characteristic of allylic acetates.

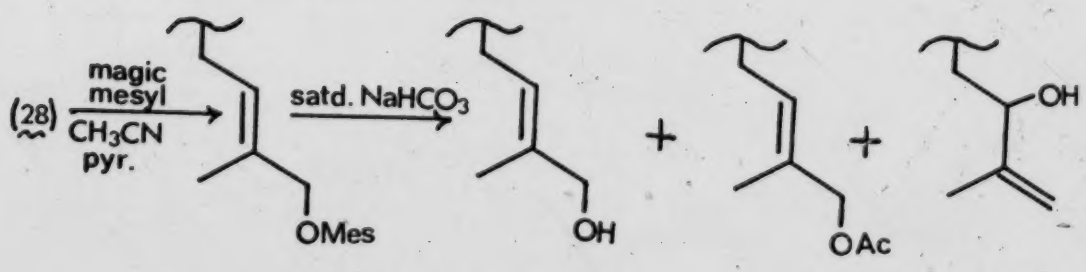
The formation of 10-chloro geranyl acetate (45) was undesirable and we immediately sought ways to avoid this obstacle. In essence, the solution lay in providing the tosyl group with a non nucleophilic leaving group. Such a derivative had been made for the mesyl group by King.[50] He treated N,N-diethyl methyl sulphonamide with methyl fluorosulphonate to obtain N,N,N-methyl diethyl methyl sulphonate. The latter proved to be a very effective mesylating reagent.



"magic mesyl"

Accordingly (10)-hydroxy geranyl acetate (28) was treated with 1 equivalent of "magic mesyl" in dry acetonitrile at -45°C, in the presence of 1 equivalent of pyridine.

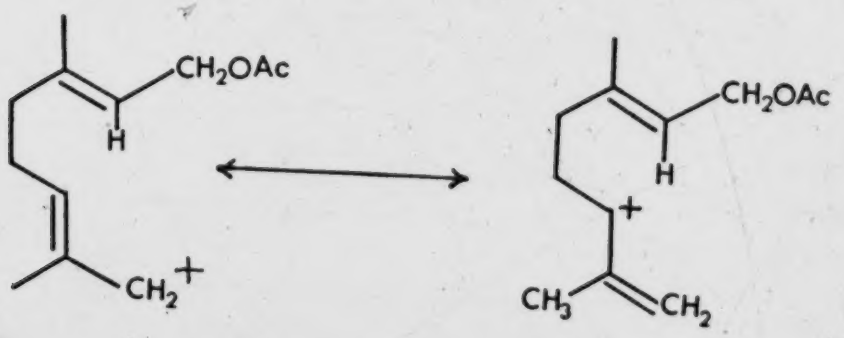
The reaction mixture was stirred for 1 hour and then gradually warmed up to room temperature. After stirring for a further hour, the reaction mixture was diluted with saturated $NaHCO_3$ and extracted with ether. The product was a mixture of three compounds. These were separated by prep dry column chromatography into two fractions. One of these fractions was quickly identified by mass spectroscopy and P.M.R. as 10-acetoxy geranyl acetate (44). The other fraction was a mixture of two compounds. These could not be separated by either G.L.C. or T.L.C. Fortunately they were recognized as isomers and shown to be 10-hydroxy geranyl acetate (28) and the allylic isomer (46).



(28) (44) (46)

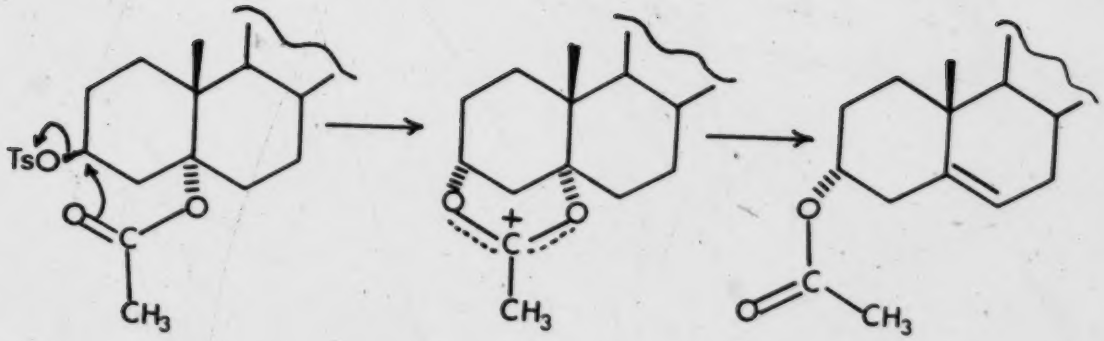
Again in this reaction, the yields were low, as the formation of (44) results in the production of polymeric material.

The ratio of (46) to (28) was 6/4. This was in keeping with the predicted stability of the carbonium ions (47) and (48).

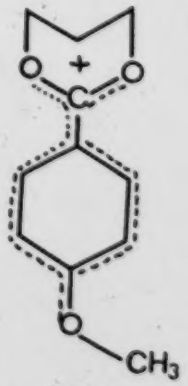


(47) (48)

The formation of the two allylic alcohols (28) and (46), along with the continual occurrence of the diacetate in the reaction products observed, was ample proof that a carbonium ion had been formed at C-10. What was obvious, however, was that this carbonium ion would not cyclize by addition to the 2,3 double bond. It had been anticipated earlier, that this addition would be facilitated by anchimeric assistance by the acetate function.[51] This is a well documented phenomenon[52] in steroids and carbohydrates, e.g.,



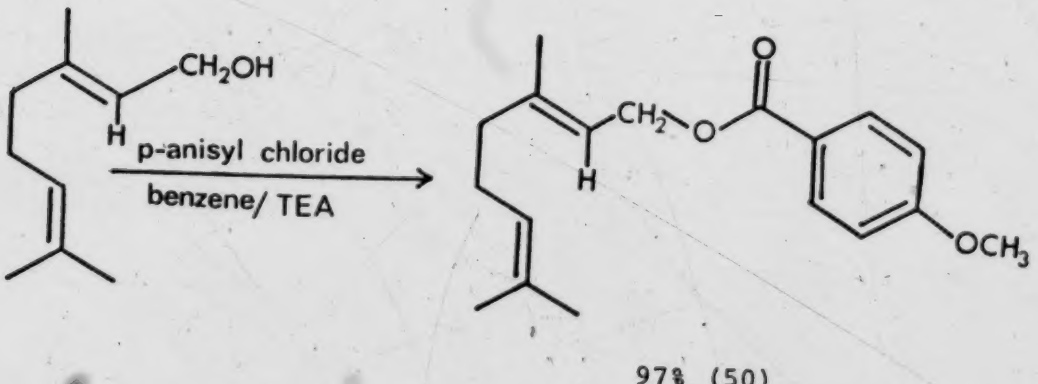
It has been shown that p-anisate esters are by far the most efficient ester group for this process.[53] This is because of unique resonance forms involved in the intermediate carbonium ion (49).



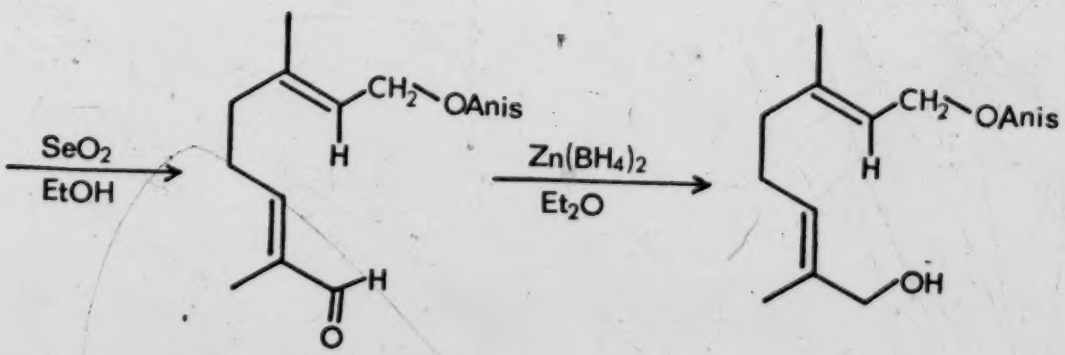
(49)

Thus before abandoning this approach we resolved to test the C-10 carbonium ion derivative of geranyl p-anisate.

Geranyl p-anisate was prepared in 97% yield by reaction of geraniol and p-anisoyl chloride in benzene using triethylamine as the base.



97% (50)



30% (51)

(52)

Selenium dioxide oxidation in ethanol of (50), under conditions similar to those for geranyl acetate (22A) gave the aldehyde (51) in 30% yield after extensive chromatography. The reaction product was not as clean or as simple as in the case of geranyl acetate. However the

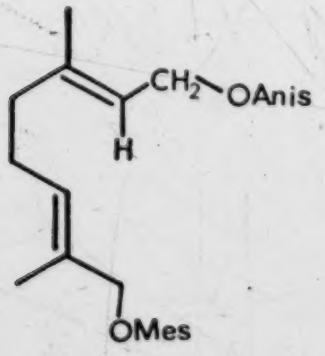
aldehyde is easily identified and isolated by prep dry column chromatography on silica gel because of its strong U.V. absorption at 254 nm. It was characterized by P.M.R. and I.R. spectroscopy. The P.M.R. spectrum indicates two olefinic protons at δ 5.5 and δ 6.35. The position of the latter confirms the geometry of the C-8,9 double bond. The strong downfield shift of C-8 proton from its regular position at δ 5.05 to δ 6.35 is indicative of the postulated configuration. An aldehyde proton appears at δ 9.3. The I.R. spectrum shows two carbonyl bands at 1690 cm^{-1} and 1710 cm^{-1}. The former is due to the $\alpha\beta$ unsaturated aldehyde.

Reduction of (51) with zinc borohydride in diethyl ether gave the allylic alcohol (52) in good yield. This compound's P.M.R. spectrum shows a hydroxymethylene at δ 3.8. This is in excellent agreement with a similar value for the 10-hydroxy geranyl acetate (28).

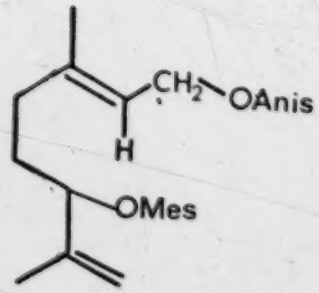
The mass spectrum of (52) was measured using chemical ionization with ammonia. The spectrum showed a base peak at m/e 322 corresponding to (M + 18). This structure was also supported by I.R. spectroscopy. The latter confirmed the presence of a hydroxy group by a broad band at 3450 cm^{-1}. Also the carbonyl absorption at 1690 cm^{-1} present in (51) was absent.

Treatment of a methylene chloride solution of (52) at -78°C with a solution of "magic mesyl" in acetonitrile

under N_2 gave the mesylate (53). This compound could be examined by evaporation of the solvents followed by neutral work-up. It was quite unstable and certainly impure. However its essential nature could be observed by P.M.R. spectroscopy. By this method the sulphonyl methyl group was observed at δ 2.9. It appeared to be a very narrow doublet. As this group could not be coupled to any other proton, the presence of two isomeric forms of the mesylate was indicated. This was confirmed by the singlet at δ 4.48 due to C-10 methylene. This integrated to only half the value of the C-1 methylene. Although it could not be definitely proven, it could be reasonably assumed that this is due to allylic isomerization of the mesyl function between C-10 and C-7, giving the structures (53) and (54).



(53)

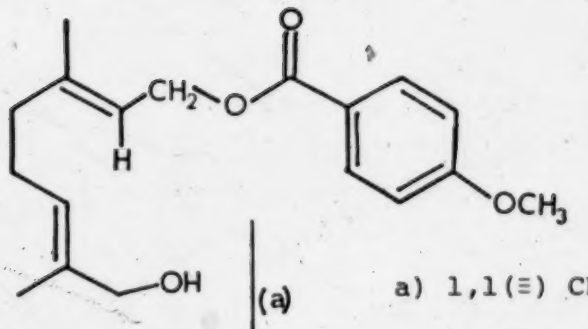


(54)

In practise, however, the mesylate was not usually isolated but instead the methylene chloride solution in

which it was prepared was diluted with a large excess of the solvent in which the solvolysis was to be studied. The solvent that was chosen for this was hexamethylphosphoramide. This is a strongly dipolar aprotic solvent and an excellent medium for ionizing reactions. Treatment of the mesylate with this solvent for 12 hours at room temperature gave upon work-up, the alcohol (52). No cyclized products could be detected. This result was always obtained despite much variation in reaction conditions with regard to time and temperature.

To confirm that a carbonium ion was indeed being formed at C-10 it was decided to add a non-nucleophilic Lewis base to the hexamethylphosphoramide solution during the solvolysis. The base chosen was perdeuteromethanol. Thus any carbonium ion formed should be immediately trapped as its perdeuteromethyl ether.

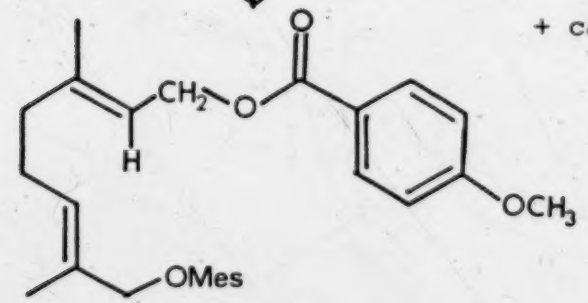


a) 1,1(≡) $CH_3CO_2\text{-}\overset{\oplus}{N}(ET)_2Me\ FSO_3^{\ominus}$

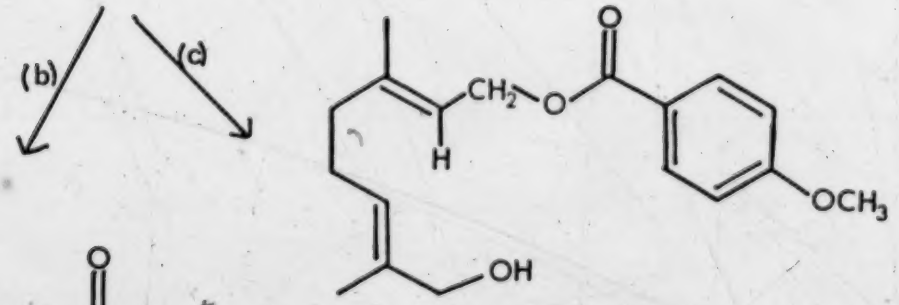
CH_2Cl_2 | CH_3CN

+ catalytic amt. pyridine

(52)



(53)



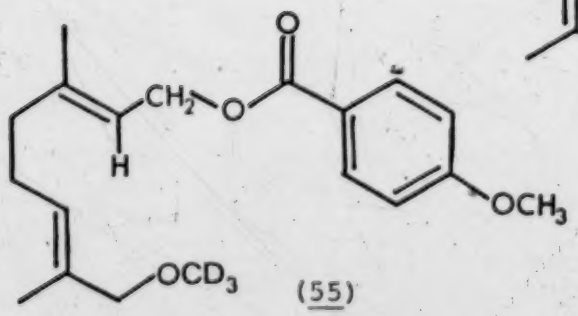
(52)

b) 1. $(Me_2N)_3PO$

2. CD_2OD

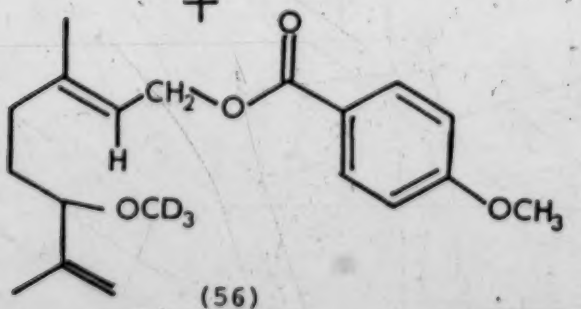
c) 1. $(Me_2N)_3PO$

2. H_2O



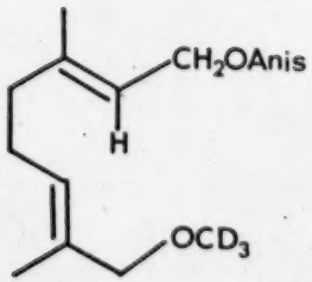
(55)

+

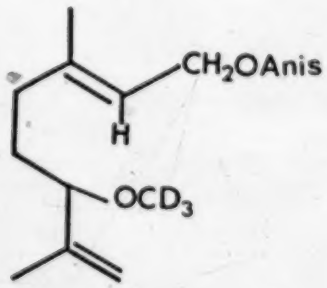


(56)

The products of this experiment were the perdeuteromethyl ethers (55) and (56). This was confirmed by chemical ionization mass spectroscopy. The mass spectrum contained a parent peak as base peak at m/e 339 (M + 18). Since the ionizing gas was ammonia this corresponds to a molecular weight of 321. Exact mass measurement also confirmed this structure. The P.M.R. spectrum showed the C-10 methylene as a singlet at δ 3.8. This value is correct for an allylic methoxymethylene group. The spectrum also confirmed the presence of allylic isomers. The terminal vinylidene protons appeared as a multiplet at δ 4.9. The ratio of (55) to (56) was 9:7.



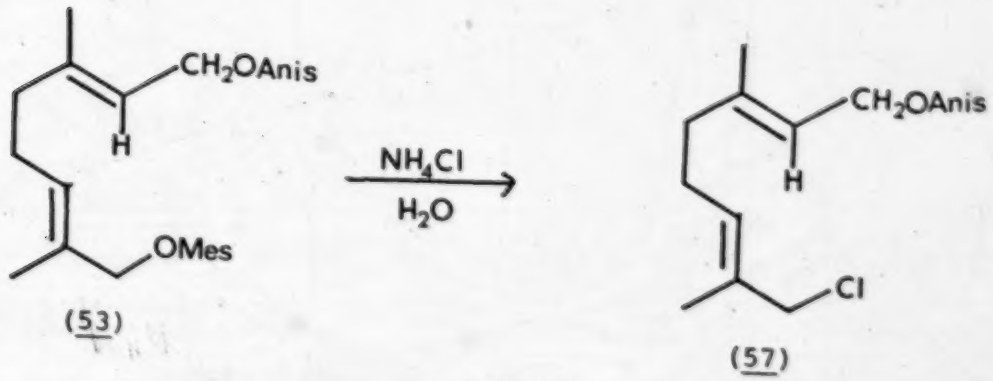
(55)



(56)

This result confirms the presence of carbonium ion character at C-7 and C-10 during the solvolysis. That no cyclization was observed can only be due to the unfavourable nature of such a reaction.

As an aside it must be mentioned that attempts to purify the mesylate (prepared in methylene chloride/ acetonitrile solvent) by aqueous work-up with saturated ammonium chloride gave the chloride (57). Substitution of ammonium perchlorate solution in the work-up procedure solved this problem and the presence of the mesylate could be detected.

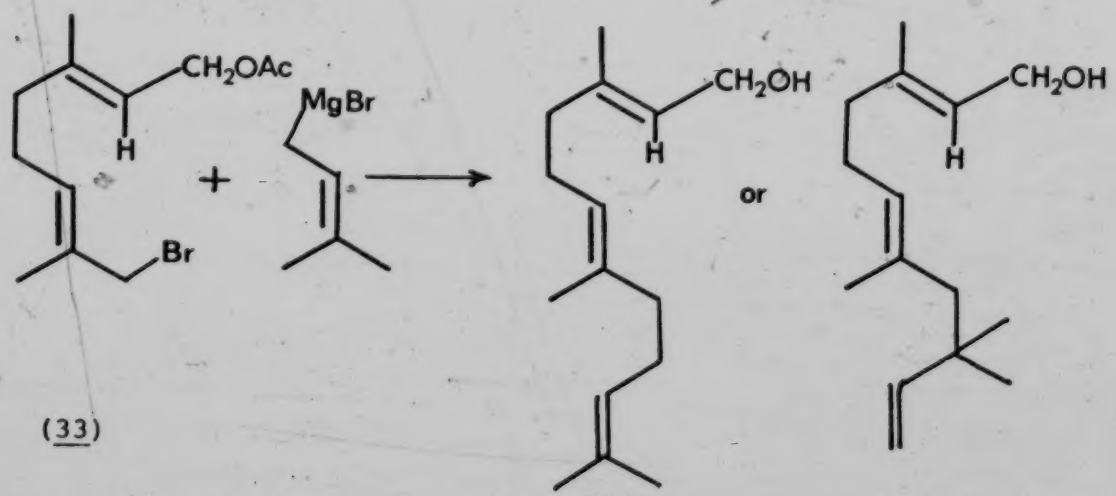


(53) (57)

The formation of the chloride (52) is excellent proof of the structure of the mesylate (53). Such a reaction for mesylates is well known.[49] The structure of the chloride was amply supported by mass spectral evidence. The mass spectrum as determined by chemical ionization with ammonia showed a base peak at m/e 340. A peak at m/e 342 which was 30% of the 340 peak confirmed the presence of chlorine. The P.M.R. spectrum showed the presence of the allylic chloromethylene as a singlet at δ 4.0.

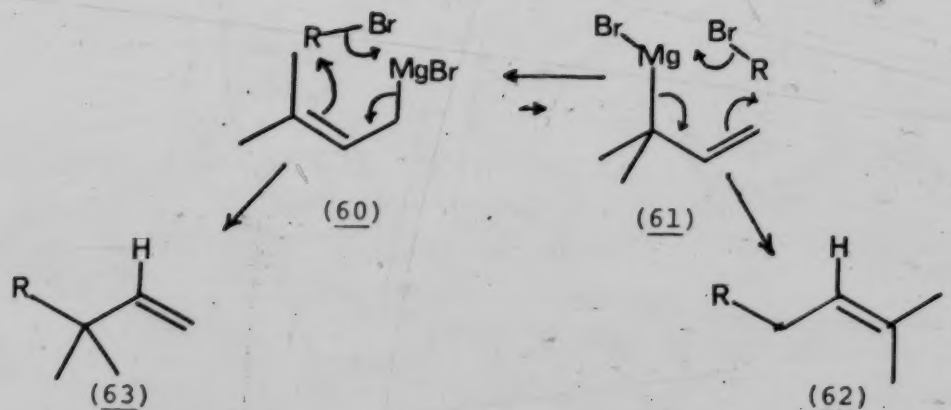
In closing this chapter one can say that the formation of cyclopentane systems by such a carbonium ion route as we proposed is not feasible.

Before abandoning this project we decided to investigate alternate uses for the C-10 functionalized geraniol derivatives synthesized. In particular, we wished to know if 10-bromogeranyl acetate (33), could be used as a substrate in homologation reactions. The reaction we chose to investigate was the coupling of allylic Grignard reagents, e.g.,



(33) (58) (59)

There are two possible products from such a reaction. Of these compounds (59) is the most likely, as this reaction is essentially a ligand exchange process.[54]



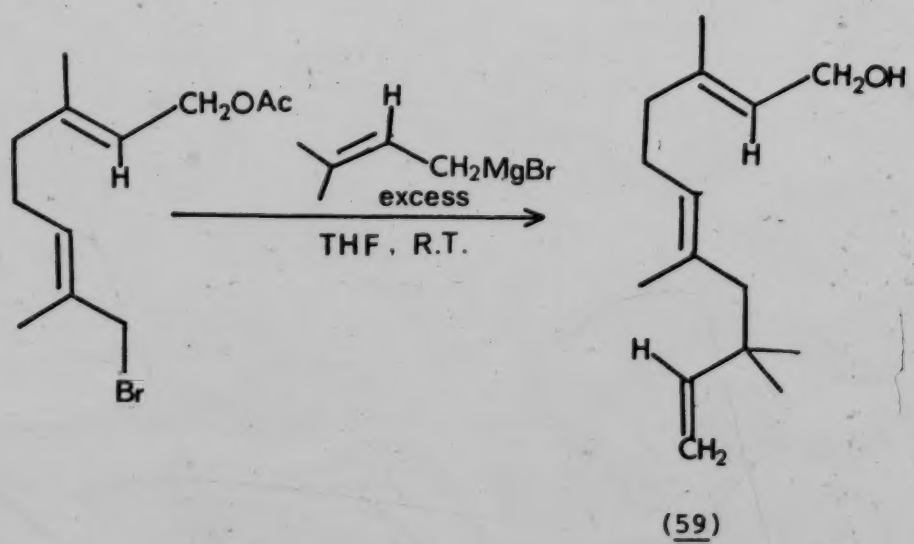
(60) (61) (63) (62)

Of the two equilibrium forms of dimethylallyl magnesium bromide the form (61) constitutes only 1%[33] and therefore the cross coupled product should be the major one. However in many similar examples of this type of reaction, the straight coupled product (58) is often produced in yields of up to 40%. The reason for this is not clear. The possibility of getting the straight coupled product was excellent inducement for trying the reaction. Even if the only product was cross coupled - it constituted a useful test for this type of reaction.

Accordingly dimethylallyl magnesium bromide was prepared. This was a difficult task as the main product of the reaction of dimethylallyl bromide and magnesium was not the Grignard reagent but the coupling product 3,3,6-triemthyl-1,5-heptadiene.

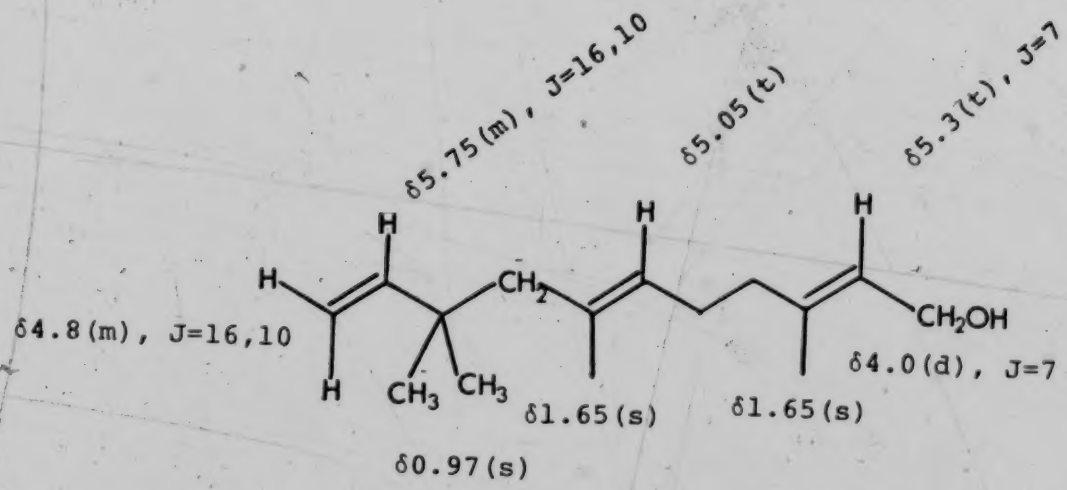
Useful yields of the Grignard reagent could only be achieved by preventing the allylic bromide from coming into contact with the Grignard reagent. Such a process was possible if a very dilute solution of allylic halide was slowly percolated through a large excess of magnesium. The latter must be activated by amalgamation with mercuric bromide so that the reaction with the halide was essentially instantaneous. This method achieved yields of the Grignard reagent of 40%. (The exact details are described in the experimental section).

Reaction of excess dimethylallyl magnesium bromide and 10-bromogeranyl acetate in THF at room temperature gave three products. These were separated by prep T.L.C. chromatography on silica gel. Only one of these compounds could be isolated pure - but it corresponded to farnesol (58) in its behaviour on T.L.C.



(59)

However, this compound was identified as the cross coupled product (59). This was done by P.M.R. spectroscopy - the results of which are outlined below.



The I.R. spectrum showed bands at 3600 cm^{-1} (s) and 980 cm^{-1} confirming the presence of a hydroxy function. High resolution mass spectroscopy confirmed the formula as $C_{15}H_{26}O$.

Postscript

In a notable series of papers Baldwin and co-workers[54] have recently shown that cyclizations such as we were proposing to carry out, are extremely unfavoured. Using a new technique called "Approach Vector Analysis" he shows that formation of five-membered rings by endo addition to a double bond is unfavourable. This is due to stereochemical factors involved in the transition state for such a reaction. In retrospect one can say that our results are in complete agreement.

EXPERIMENTAL

Melting points were determined on a Fischer-Johns hot-stage melting point apparatus and are uncorrected.

Infrared spectra were recorded on a Perkin-Elmer Model 241 dual grating spectrophotometer.

Proton magnetic resonance spectra were measured using a Varian Associates Model HR-100 spectrometer, interfaced in the Fourier mode to a Digilab FTS/NMR 3 data system.

Carbon magnetic resonance spectra were measured using a Bruker HFX-90 spectrometer or a Bruker WP-60 spectrometer. In either case the Fourier mode was used.

Mass spectra were recorded on an A.E.I. Model MS-9 or an A.E.I. Model MS-50 mass spectrometer. Chemical ionization spectra were recorded on an A.E.I. Model MS-12 mass spectrometer using ammonia as an ionizing gas.

U.V. spectra were determined on a Cary Model 15 U.V. and visible spectrometer.

Gas chromatography was performed on a Hewlett-Packard 5700/A chromatograph using a flame ionization detector.

Micro analyses were performed by the Microanalytical Laboratory of this department.

Thin layer chromatography was done generally on micro plates (75 x 25 mm) using Merck Silica Gel G (type 60), and using General Electric Type 118-2-7 Electronic Phosphor as a fluorescent indicator. Plates were examined with

U.V. light at a wavelength of 254 n.m.

Dry column chromatography was performed on Woelm Silica Gel (for Dry Column Chromatography - Activity II or III), in 1" diameter nylon tubing.

Purification of Geraniol

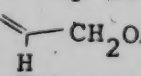
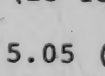
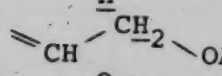
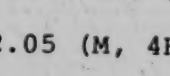
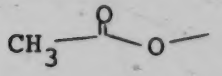
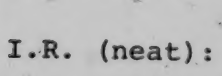
Calcium chloride (anhydrous, finely powdered, 250 gms) was added to a solution of geraniol (300 gms) in 1200 mls of hexane. The mixture was stirred vigourously using a magnetic stirrer to prevent caking of the calcium chloride, and simultaneously cooled to -40°C. After about 10 minutes the geraniol-$CaCl_2$ complex formed and stirring was no longer possible. The mixture was kept at -40°C for 1 hour. It was filtered while cold as quickly as possible through a large Buchner funnel. The precipitate was collected and washed with cold hexane (2 x 100 mls). The Buchner funnel was then covered with a latex rubber membrane and the vacuum was applied for a further 10 minutes. The membrane allowed the last traces of the hexane to be removed without exposing the precipitate to the atmosphere.

The precipitate was hydrolysed using a mixture of water and ether. The ether layer was removed, washed with water and dried with saturated NaCl solution and $MgSO_4$. Evaporation of the ether, followed by vacuum treatment to remove final traces, yielded pure geraniol.

Typical purity is 95% or greater. Yield varies with geraniol content of starting material.

Preparation of Geranyl Acetate (22A)

Sodium acetate (anhydrous, 41 gms, 0.5 moles) was added to a solution of geraniol (77 gms, 87 mls, 0.5 moles) in benzene (500 mls). The solution was stirred and acetic anhydride (61 gms, 56 mls, 0.6 moles) was slowly added. The mixture was refluxed for 4 hours.

After cooling, the mixture was extracted with saturated $NaHCO_3$ solution (200 mls) and saturated NaCl (200 mls) and dried over $MgSO_4$. Removal of the benzene by concentration under vacuum followed by spinning band distillation of the residue yielded pure geranyl acetate (22A) (81 gms, 83%): bp 123-124°C (15 Torr)) P.M.R. ($CDCl_3$): δ 5.3 (t, 1H, ), δ 5.05 (M, 1H, ), δ 6.5 (d, 2H, ), δ 2.05 (M, 4H, ), δ 1.95 (S, 3H, ), δ 1.65 (M, 9H, ).

I.R. (neat): 1740 cm^{-1} (C=O), 1675 cm^{-1} (C=C)

Mass Spectrum: 196 (M, absent), 154.1358 (M - 42, $C_{10}H_{18}O$) (0.17%), 136.1249 (M - 60, $C_{10}H_{16}O$) (16%), 121.1014 (M - 75, $C_9H_{13}O$) (14%), 93.0699 ($C_7H_9^+$) (31%), 69.0706 ($C_5H_9^+$) (100%).

Selenium Dioxide Oxidation of Geranyl Acetate

A solution of geranyl acetate (100 gms, 0.51 moles) in 95% ethanol (500 mls) was heated to reflux. A solution of selenium dioxide (56 gms, 0.5 moles) in 95% ethanol (500 mls) was slowly added while maintaining reflux. Addition was complete after 1/2 hour and the solution was refluxed for a further hour. It was then cooled and decanted from the precipitated selenium metal. After filtration it was concentrated under vacuum until no further ethanol could be removed. The residue was dissolved in ether (400 mls) and washed with 10% Na_2CO_3 solution. At this stage there was considerable effervescence.

The ether layer was washed again with 10% aqueous Na_2CO_3 solution, finally with saturated aqueous NaCl solution and dried over $MgSO_4$. Removal of the ether gave a yellow oil (116 gms, 90% approximately).

Gas chromatography on a FFAP column (5' x 1/8") programmed from 150→200 @ 4° min. indicated 11% starting material (22A), 51% aldehyde (27) and 38% alcohol (28). A sample of pure aldehyde (27), was obtained by dry-column chromatography on Woelm Silica Gel (Activity II) using pure methylene chloride as elutant. The aldehyde is easily located on the nylon column by its quenching of the 254 nm fluorescence of the indicator.

P.M.R.: δ 6.42 (t, 1H, $C\underline{H}=\langle^{CHO}_{Me}$), δ 5.37 (t, 1H, $=C\underline{H}-CH_2OAc$), δ 2.35 (m, 4H, $C\underline{H}_2-C=C$) δ 2.0 (s, 3H, $C\underline{H}_3-C(=O)-O$) δ 1.75 (broad s, 6H, $C\underline{H}_3-C=C$)

I.R.: 1740 cm^{-1} ($-CO_2Me$), 1690 (C=O), 1645 (C=C).

Mass Spectrum:

A) Chemical ionization with ammonia: 228 (M + 18)

B) Electron bombardment: 210 (M, absent), 150.1043 (M - 60), $C_{10}H_{14}O$, 75%), 135.0811, (M - 75, $C_9H_{11}O$, 28%), 122.1019 (M - 89, C_9H_{13}, 36%), 107.0858 ($C_8H_{11}O$, 18%), 93.0701 (C_7H_9, 23%), 91.0545 (C_7H_7, 12%), 85.0646 (C_5H_9O, 42%), 84 (C_5H_8O, 100%), 82.0415 (C_5H_6O, 26%), 79.0544 (C_6H_7, 13.6%), 68.0626 (C_5H_8, 14%), 67.0551 (C_5H_7, 22%).

Oxime of 10-oxogeranylacetate (29)

A mixture of hydroxylamine hydrochloride (0.5 gms), aldehyde (29) (0.5 gms) and pyridine (0.5 mls) in ethanol (5 mls) was refluxed for 1 hour. The solution was cooled and the alcohol removed by concentration under vacuum. Addition of water (5 mls) to the residue gave a yellow oil. Various attempts to induce crystallization in this oil led to failure.

Semicarbazone of 10-oxogeranylacetate (30)

Semicarbazide hydrochloride (1 gm) and crystallized sodium acetate (1.5 gms) were dissolved in water (10 mls).

The solution was stirred vigorously and the aldehyde (27) (0.75 gms) was slowly added. Reaction was complete within 5 minutes, by which time the semicarbazone had precipitated out as white crystals. These were filtered off and recrystallized from 95% ethanol and dried in a vacuum pistol (yield 1 gm).

M.P. 121-122°C

Analysis: Calc. for $C_{13}H_{21}O_3N_3$; C, 58.41; H, 7.92; N, 15.72. Found; C, 58.36; H, 7.90; N, 15.88.

P.M.R. (CCl_4/$DMSOD_6$): δ 10.30 (s, 1H, $=N-\underline{NH}-C(=O)$), δ 7.45 (s, 1H, $-\underline{CH}=N-$), δ 6.10 (bs, 2H, $-\underset{\underset{O}{\|}}{C}-\underline{NH_2}$), δ 5.2-5.7 (t, t, 2H, $C=\underline{CH}$), δ 4.52 (d, 2H, $=C-\underline{CH_2}-OAc$), δ 2.2 (bm, 4H, $C=C-\underline{CH_2}-$), δ 2.0 (s, 3H, $\underline{CH_3}-CO_2-$), δ 1.8 (s, s, 6H, $\underline{CH_3}-C=C$).

I.R. (nujol): 1730 cm^{-1}; (C=O ester), 1690 cm^{-1}; (C=O amide), 1670 cm^{-1}; (C=C).

Mass spectrum: 267 (M, absent), 187 (7%), 169 (12%), 153 (15%), 149 (16%), 141 (20%), 139 (18%), 127 (37%), 125 (31%), 111 (37%), 109 (57%), 99 (57%), 95 (57%), 93 (49%), 91 (29%), 87 (60%), 81 (100%), 71 (79%), 67 (56%).

Attempted cleavage of semicarbazone (30)

A solution of thallium (III) nitrate trihydrate (1.0 gms, 2.24 mmoles) in methanol (20 mls) was prepared and cooled to 0°C in an ice bath. To it was added a solution of semicarbazone (30) (0.5 gms, 2.28 mmoles) in methanol (8 mls). This mixture was stirred continuously for 15 minutes, by which time a white precipitate of thallium (I) nitrate had formed. The mixture was filtered and the filtrate acidified with 10 drops of 0.1 molar aqueous H_2SO_4. It was then diluted with ether (100 mls) and washed with saturated aqueous $NaHCO_3$ solution, water (100 mls), and saturated aqueous NaCl solution, and then dried over $MgSO_4$. Concentration of the solution under vacuum gave an oil (0.5 gms). Examination of this oil by T.L.C. (silica gel, chloroform methanol) indicated at least four components, one which was the original semicarbazide and two were carbonyl containing. It was obvious that the side reaction concerning double bonds mentioned in ref. 25 had occured and no further investigation was made.

Reduction of 10-oxogeranylacetate (27) by Sodium Borohydride

Sodium borohydride (7 gms, 0.185 moles) was dissolved in ice-cold dried diglyme (250 mls). Then a mixture of aldehyde (27) and alcohol (28) (60 gms) in diglyme (100 mls) was slowly added. When addition was complete the solution

was stirred for a further 8 hours at room temperature. It was then diluted with ether (1000 mls) and washed with saturated aqueous $NaHCO_3$ (200 mls), 10% aqueous Na_2CO_3 (200 mls), water (200 mls) and finally saturated NaCl solution (200 mls). It was then dried over $MgSO_4$ and concentrated under vacuum to give a yellow oil (96 gms). This still contained diglyme. It was fractionally distilled at 0.07 (Torr) and the fraction B.P. 117-125°C was collected. This was a mixture of two compounds. Gas chromatography on FFAP (8' x 1/8") programmed for 150-200°C @ 4°C/min showed two components in the ratio 2:1. The minor component had the shorter retention time.

P.M.R. ($CDCl_3$): major component - δ 5.4 (t, 2H, $C=C<\underline{H}$), δ 4.6 (d, 2H, $-C\underline{H}_2-OAc$), δ 4.0 (s, 2H, $-C\underline{H}_2-OH$), δ 2.8 (s, 1H, $-O\underline{H}$, D_2O), δ 2.1 (m, 4H, $C=C-C\underline{H}_2$), δ 2.0 (s, 3H, $C\underline{H}_3-\overset{O}{\overset{\|}{C}}-O-$), δ 1.7 (s, 6H, $C\underline{H}_3-C=C$).
minor component - δ 5.4 (t, 2H, $C=C\underline{H}-$), δ 4.6 (d, 2H, $-C\underline{H}_2-OAc$), δ 3.48 (d, 2H, $-C\underline{H}_2-OH$), δ 2.8 (s, 1H, $-O\underline{H}$, D_2O), δ 2.1 (m, 2H, $C=C-C\underline{H}_2$), δ 2.0 (s, 3H, $C\underline{H}_3-CO-O-$), δ 1.7 (s, 3H, $C\underline{H}_3-C=C$), δ 1.2-1.8 (m, 5H, $-C\underline{H}_2-$, $-C\underline{H}-$), δ 0.9 (d, 3H, $C\underline{H}_3-CH-$).

Preparation of Zinc Borohydride

A suspension of anhydrous zinc chloride (34 gms, 0.25 moles) and sodium borohydride (19 gms, 0.5 moles) in anhydrous ether was vigorously stirred for 48 hours.

The resulting mixture was filtered from the precipitated sodium chloride in a glove box under a dry N_2 atmosphere. The clear filtrate was then standardized by volumetric estimation of the released hydrogen when a quantity of the solution was mixed with methanol. By this process it was found to be 0.2 M (in zinc).

Zinc Borohydride Reduction of Aldehyde (27)

A solution of crude aldehyde (27) (9 gms, 0.043 moles) in ether (100 mls) was prepared. This solution was cooled to 0°C and stirred while a solution of 0.2 M $Zn(BH_4)_2$ in ether (120 mls, 0.024 moles) was slowly added. When addition was complete the mixture was allowed to warm to room temperature and stirred for 1 hour. The ether solution was shaken in a separatory funnel with pH 7 buffer (100 mls) and the ether layer drawn off. This was washed with water (100 mls) and saturated aqueous NaCl solution (100 mls) and dried over Na_2SO_4. Removal of the ether by vacuum concentration gave an oil (8.1 gms, 88%).

P.M.R. (CCl_4): δ 5.35 (t, 2H, C=C$\underline{H}$—), δ 4.53 (d, 2H, C$\underline{H}_2$—OAc), δ 3.88 (s, 2H, C$\underline{H}_2$—OH), δ 3.4 (s, 1H, —O$\underline{H}$, D_2O), δ 2.1 (m, 4H, C$\underline{H}_2$—C=C), δ 2.0 (s, 3H, C$\underline{H}_3$—CO—O—), δ 1.73 (s, 3H, C$\underline{H}_3$—C=C), δ 1.63 (s, 3H, C$\underline{H}_3$—C=C).

I.R. (neat): 3400 cm^{-1} broad (—O— H), 1730 cm^{-1} (C=O), 1660 cm^{-1} shoulder (C=C), 1230 cm^{-1} (C—O).

Mass spectrum: chemical ionization (NH_3), 230 (M + 18, 100%). electron bombardment, 152 (M - 60, $C_{10}H_{16}O$, 5%), 150 (M - 62, $C_{10}H_{14}O$, 31%), 149 ($C_8H_5O_3$, 32%), 134 ($C_{10}H_{14}$, 33%), 121 (C_9H_{13}, 25%), 119 (C_9H_{11}, 21%), 94 (C_7H_{10}, 22%), 93 (C_7H_9, 32%), 85 (C_5H_9, 28%), 84 (C_5H_8, 100%), 79 (C_6H_7, 22%), 68 (C_5H_8, 75%), 67 (C_5H_7, 42%).

Preparation of anhydrous magnesium chloride

Magnesium chloride hexahydrate (50 gms, 0.25 moles) was placed in a large flask fitted with a dropping funnel and a reflux condenser. Thionyl chloride (450 gms, 274 mls, 3.8 moles) was slowly added *via* the dropping funnel. When addition was complete, the mixture was refluxed for 1 hour and then the excess thionyl chloride was removed by concentration under vacuum. The residue was heated at 90°C for 8 hours at 0.5 Torr. Yield 24 gms (quant.)

Manganese Dioxide Oxidation of Alcohol (28)

A mixture of alcohol (28) (2 gms, 0.0094 moles), manganese dioxide (4 gms, 0.046 moles) (activated, prepared by the method of Attenburrow, see ref. 31) and methylene chloride (100 mls) was stirred under N_2. Examination of the supernatant by G.L.C. (OV-17, 150-200°C

@ 8°C/min) after 12 hours revealed a mixture of the aldehyde (27) (85%) and alcohol (28) (15%). Within 24 hours the aldehyde constituted 95% of the mixture. Filtration and concentration *in vacuo* gave the pure aldehyde (27). Yield 1.9 gms (94%).

Preparation of bromoacetate (33)

Phosphorous tribromide (6 gms, 0.022 moles) was slowly added to a solution of alcohol (28) (7 gms, 0.033 moles) in dry ether (125 mls) at 0°C with stirring. When addition was complete the reaction mixture was stirred for 8 hours at 0°C. It was then worked-up by hydrolysis with pH 7 buffer (100 mls) and the ether layer washed with water and aqueous saturated NaCl solution and dried over Na_2SO_4. Removal of the ether *in vacuo* gave the bromoacetate (33) (4.35 gms, 46%).

The latter was further purified by dry column chromatography on silica gel using methylene chloride as solvent. Pure bromoacetate (33) was isolated. Yield 3.9 gms (43% overall). Analysis: Calc. for $C_{12}H_{19}O_2Br$, C, 52.38; H, 6.96; Br, 29.04. Found C, 52.81; H, 7.04; Br, 29.75. Note, this compound was unstable.

P.M.R. (CCl_4): δ 5.52 (t, 1H, C=C<u>H</u>), δ 5.3 (t, 1H, C=C<u>H</u>), δ 4.55 (d, 2H, C<u>H</u>$_2$—OAc), δ 3.85 (s, 1H, C<u>H</u>$_2$—Br), δ 2.1 (m, 4H, C<u>H</u>$_2$—C=C), δ 2.0 (s, 3H, C<u>H</u>$_3$—C(=O)—O—), δ 1.75 (s,

(s, 3H, $C\underline{H}_3-C=C$), δ 1.7 (s, 3H, $C\underline{H}_3-C=C$).

I.R. ($CHCl_3$, 0.5 mms): 1730 (C=O), 1670 (C=C), 1270 (CH_2-Br).

Mass spectrum: chemical ionization with ammonia, 292 (M + 18, 100%), 294 (M + 18, 98%); electron bombardment; 217 (M + 2 - 60, 1%), 215 (M - 60, 1%), 149 (3%), 147 (3%), 135 (M - 60 - Br, 60%), 121 (10%), 117 (10%), 93 (60%), 85 (40%), 83 (60%), 81 (40%), 69 (100%), 68 (90%), 67 (80%).

Preparation of bromoacetate (33) using PBr_3 and LiBr.

Phosphorous tribromide (0.7 gms, 0.25 mls, 0.026 moles) was slowly added to a stirred mixture of lithium bromide (1.0 gms, 0.0135 moles) and alcohol (28) (1.5 gms, 0.007 moles) in ether (100 mls) at 0°C. Stirring was continued at 0°C for 24 hours. Work-up was identical with the previously described sequence and yielded pure bromoacetate (33). Yield 1.7 gms (88%).

Effect of Methylmagnesium Iodide on the Bromoacetate (33)

Methylmagnesium iodide in THF (4 mls of 1.84 M, 7.36 mmoles) was added to a solution of bromoacetate (33) (500 mgms, 1.82 mmoles) in dry T.H.F. (50 mls) at 0°C. The solution was stirred at 0°C for 7 hours. Analysis of a small aliquot (T.L.C., silica gel, chloroform, methanol) indicated the complete disappearance of the bromoacetate

(33). The reaction mixture was diluted with ether (200 mls) and hydrolysed with pH 7 buffer (200 mls). The ether layer was drawn off, washed with water and saturated NaCl solution and dried over Na_2SO_4. Concentration *in vacuo* gave an oil (260 mgms, 85%). On T.L.C. (silica gel, chloroform/methanol) this oil gave a single spot (R.F. 0.55) identical with that of geraniol. G.L.C. (FFAP, 10' x 1/8", 110-200 @ 4°C/min) indicated the presence of two compounds in the ratio 9:1. Compared to geraniol both compounds have longer retention times, with the major of the two being the longest.

Major Component (35A)

P.M.R. (CCl_4): δ 5.32 (t, 1H, C=C$\underline{H}$), δ 5.05 (m, 1H, C=C$\underline{H}$), δ 4.0 (d, 2H, C$\underline{H}_2$—OAc), δ 2.0 (m, 6H, C$\underline{H}_2$—C=C), δ 1.6 (s, 3H, C$\underline{H}_3$—C=C), δ 1.55 (s, 3H, C$\underline{H}_3$—C=C), δ 0.95 (t, 3H, C$\underline{H}_3$—CH_2).

G.L.C./Mass spectrum: 168 (M , absent), 150 (M - 18, <1%), 121 (10%), 93 (85%), 91 (10%), 83 (15%), 79 (20%), 77 (15%), 67 (20%), 55 (100%), 41 (80%).

Minor Component (35B)

P.M.R. (CCl_4): δ 5.32 (t, 1H, C=C$\underline{H}$), δ 4.52 (d, 2H, C=C$\underline{H}_2$), δ 4.0 (d, 2H, C$\underline{H}_2$—OAc), δ 2.0 (m, 4H, C$\underline{H}_2$—C=C), δ 1.6 (s, 3H, C$\underline{H}_3$—C=C), δ 1.55 (s, 3H, C$\underline{H}_3$—C=C), δ 1.0 (d, 3H, C$\underline{H}_3$—CH).

G.L.C./Mass spectrum: 168 (M, absent), 166 (M - 2), <1%), 150 (M - 18, >1%), 135 (10%), 121 (15%), 107 (20%), 95 (2%), 94 (20%), 93 (20%), 82 (25%), 81 (50%), 79 (30%), 69 (25%), 67 (30%), 55 (35%), 53 (30%), 41 (100%).

Mixture 90% (35A) and 10% (35B)

I.R. ($CHCl_3$, 0.5 mm): 3600 cm^{-1}, s, (O-H); 1670 cm^{-1}, w,(C=C); 1655 cm^{-1}, w,(C=C); 1645, w,(C=C);

Mass spectrum: 168.1514 (M, $C_{11}H_{20}O$, 3%), 150.1407 (M - 18, $C_{11}H_{18}$, 8%), 121.1017 (C_9H_{13}, 14%), 93.0702 (C_7H_9, 31%), 83.0860 (C_6H_{11}, 50%), 82.0780 (C_6H_{10}, 19%), 81.0702 (C_6H_9, 13%), 79.0547 (12%), 67.0551 (C_5H_9, 23%), 55.0561 (C_4H_7, 100%).

Reduction of Bromoacetate (33)

Diisobutylaluminum hydride (25% in benzene, 10 mls, 17.6 mmoles) was added to a solution of bromoacetate (33) (2.2 gms, 8 mmoles) in Skellysolve B (50 mls) at room temperature under N_2. The solution was stirred for 5 hours. It was hydrolysed with aqueous pH 7 buffer (100 mls). The organic layer was drawn off, washed with water, and dried over Na_2SO_4. Concentration *in vacuo* gave an oil (1.25 gms, 67%).

P.M.R. (CCl_4): δ 5.55 (m, 1H, C<u>H</u>=C), δ 5.35 (t, 1H, C<u>H</u>=C), δ 4.05 (d, 2H, C<u>H</u>$_2$—OAc), δ 3.90 (s, 2H, C<u>H</u>$_2$—Br),

δ 3.25 (b.s, 1H, —O$\underline{H}$), δ 2.1 (m, 4H, C$\underline{H}_2$—C=C), δ 1.75 (s, 3H, C$\underline{H}_3$—C=C), δ 1.65 (s, 3H, C$\underline{H}_3$—C=C).

I.R. (neat): 3350 cm^{-1}, broad, (O—H); 1670 cm^{-1}, w, (C=C); 1085 cm^{-1}, s, (C—O).

Mass spectrum: chemical ionization with ammonia; 250 (M^+ + 18, 90%), 232 (M^+, 85%).

Reaction of geraniol and allyl magnesium bromide

Allyl magnesium bromide (in $^nBu_2O$, 250 mls of 0.25 M, 0.062 moles) was added to a solution of geraniol (4 gms, 0.027 moles) in $^nBu_2O$ (10 mls) under N_2. The stirred solution was refluxed for 24 hours. G.L.C. analysis of an aliquot (FFAP, 10' x 1/8", 110-200°C @ 4°C/min) indicated a mixture of two compounds in the ratio 3:1. The minor component was geraniol. The reaction mixture was cooled and extracted with pH 7 buffer. It was then washed with water, saturated aqueous NaCl solution and dried over Na_2SO_4. The $^nBu_2O$ was removed by concentration under high vacuum. The residue was a pale refractive liquid (3.6 gms). Two grams of this liquid were chromatographed on 180 gms of Woelm Silica Gel (Grade II for dry column chromatography) using methylene chloride as solvent. The desired compound ran with the solvent front, and

was recovered as a clear liquid (750 mgms). This liquid was then subjected to molecular distillation at 10 microns to give a clear colourless liquid (500 mgms).

N.M.R. (CCl_4): δ 6.0-5.4 (m, 3H ?), δ 5.15-4.65 (m, 6H ?), δ 2.0 (m, 6H), δ 1.6 (s, 6H, $CH_3-C=C$), δ 1.55 (s, 6H, $CH_3-C=C$), δ 1.2 (m, 1H), δ 0.95 (s, 6H, CH_3-C).

I.R. (neat): 3060 cm^{-1}, w, (C—H); 2900-2840 cm^{-1}, s, (C—H); 1645 cm^{-1}, m, (C=C); 910 cm^{-1}, s, ($-CH=CH_2$).

Mass spectrum: 274.2661 (M, $C_{20}H_{34}$, 5%), 260.2499 ($C_{19}H_{32}$, 10%), 258.2354 ($C_{19}H_{30}$, 9%), 178 (12%), 176 (11%), 136 (13%), 121 (18%), 109 (50%), 107 (45%), 93 (80%), 91 (60%), 81 (90%), 69 (100%), 67 (30%), 55 (30%).

Attempted Grignard cyclization of bromoalcohol (34)

Methylmagnesium iodide (1.2 mls of 1.84 M in benzene/THF, 2.2 mmoles) was added to a stirred solution of the bromoalcohol (34) (500 mgms. 2.16 mmoles) in T.H.F. (50 mls) at 0°C under N_2. After stirring for 10 minutes magnesium powder (100 mgms, 4.1 mmoles) was added and the mixture stirred at room temperature for 12 hours. The reaction mixture was decanted from the unreacted magnesium and diluted with ether (150 mls) and hydrolysed with pH 7 buffer (100 mls). The ether layer was recovered and washed with water and dried over Na_2SO_4. Concentration *in*

vacuo gave an oil (~ 300 mgms). Examination of this oil by T.L.C. (silica gel, chloroform/methanol) indicated a plethora of components. However an odor of lemons was distinctly apparent when the plate was examined. One spot (R.F. 0.6) was slightly yellow to the naked eye and strongly quenched U.V. radiation at 254 nm. Comparison of this spot with citral on T.L.C. yielded identical R.F. values. This spot was isolated by prep. T.L.C.. Comparison of the infrared spectrum with that of citral proved them to be identical.

N.M.R. (CCl_4): δ 9.9 (d, 1H, C$\underline{H}$O), δ 5.78 (d, 1H, C=$\underline{H}$C−CHO), δ 5.0 (m, 1H, C=C$\underline{H}$), δ 4.65 (s, 2H, C=C$\underline{H}_2$), δ 2.3-1.9 (7H), δ 1.68 (s, 3H, C$\underline{H}_3$−C=C), δ 1.6 (s, 3H, C$\underline{H}_3$−C=C).

Reaction of geraniol with ^{n}Butyl lithium and tetramethylethylene diamine

^{n}BuLi (10 mls of 1.6 M, 16 mmoles) was added to a solution of geraniol (1 gm, 3.9 mmoles) and TMEDA (0.745 gms, 6.4 mmoles) in dry T.H.F. (100 mls) at room temperature under N_2. The solution was stirred at room temperature for 12 hours. Analysis by G.L.C. (FFAP, 10' x 1/8", 110-200°C @ 4°C/min) indicated a new compound with a shorter retention time than geraniol. It constituted 25% of the mixture, the rest being geraniol. The reaction

mixture was hydrolysed with pH 7 buffer (100 mls) and then diluted with ether. The ether layer was collected, washed with water, and saturated aqueous NaCl solution and dried over Na_2SO_4. Concentration *in vacuo* gave an oil, which was immediately chromatographed on silica gel (Woelm, Dry Column, Grade II) with methylene chloride. The new component ran near the solvent front and was collected with ease.

Mass spectrum: 330 (M , <1%), 278 (<1%), 250 (<1%), 222 (8%), 207 (10%), 179 (15%), 123 (25%), 109 (25%), 97 (15%), 95 (18%), 83 (12%), 81 (20%), 69 (100%).

A similar procedure was used to investigate the reaction of geraniol with MeLi and ^{n}BuLi in the absence of TMEDA.

Attempted photocyclization of aldehyde (27)

10-Oxogeranyl acetate (27) (1 gm, 4.5 mmoles) was dissolved in hexane (200 mls) and irradiated with a medium pressure mercury U.V. light (Hanovia) using a pyrex filter. The progress of the reaction was monitored by G.L.C. (OV - 22, 18" x 2 mm/ 120-200°C @ 4° min). No change could be detected after 1 week of irradiation.

This reaction was repeated under identical conditions using trifluoroethanol (200 mls) as solvent. On this occasion geranyl acetate which was present as an impurity (<5%) in the aldehyde (27) disappeared over the course

of 2 weeks. The aldehyde remained unchanged.

Photochemical reaction of geranyl acetate

Geranyl acetate (2 gms, 10 mmoles) and benzophenone (100 mgms, 0.55 mmole) was dissolved in trifluoroethanol (100 mls) and irradiated with a medium pressure mercury U.V. lamp (Hanovia) and a pyrex filter. After 1 week G.L.C. analysis (OV - 22, 18" x 3 mm, 120-200 @ 4° min) indicated that 60% of the geranyl acetate had reacted. The new product appeared as a broad decomposition peak with a longer retention time than geranyl acetate. T.L.C. analysis (silica gel, chloroform/methanol) showed the new compound as a double spot, more polar than geranyl acetate. It was isolated by prep. T.L.C. (silica gel, chloroform/methanol) as a light yellow oil with a penetrating odor which tended to induce dizziness.

P.M.R. (CCl_4): δ 5.3 (t, 1H, C=C$\underline{H}$), δ 4.5 (d, 2H, $C\underline{H}_2-OAc$), δ 3.77 (q, 2H, J = 9 Hz, $CF_3-C\underline{H}_2-O-$) δ 2.0 (s, 3H, $C\underline{H}_3-CO-O$), δ 1.72 (s, 3H, $C\underline{H}_3-C=C$), δ 1.18 (s, 6H, $(\underline{CH}_3)_2C-O-CH_2-CF_3$).

I.R. (neat): 1742 cm^{-1}, (—OAc); 1230 cm^{-1}, (—OAc); 1155 cm^{-1} (CF_3-).

Mass spectrum: 296 (M, not found), 294 (M - 2, <1%), 252.1328 (4%, $C_{12}H_{19}O_2F_3$), 237 (3%), 234.1237 (4%, $C_{12}H_{17}OF_3$),

173 (11%), 172 (10%), 141.0525 (100%, $C_5H_8OF_3$), 140 (7%), 126 (10%), 111.0805 (40%, $C_7H_{11}O$), 110 (10%), 109 (10%), 93 (30%), 83 (30%), 81 (30%), 75 (30%), 73 (30%), 71 (30%).

F^{19} M.R. ($CDCl_3$) external CF_3CO_2H, -7.47 PPM. (T, 3F, H = 8.6 Hz), -7.72 (T, 3F, 8.6 Hz), ratio -7.47/-7.72 $\simeq$ 10/1.

Treatment of hydroxyacetate (28) with p-Toluenesulphonic acid

A solution of hydroxyacetate (28) (1 gm, 4.76 mmoles) and p-toluenesulphonic acid monohydrate (150 mgms, 0.79 mmoles) was prepared in benzene and refluxed overnight in conjunction with a Dean and Stark water collection device. The purpose of the latter was to collect the water of crystallization of the toluenesulphonic acid plus any water produced in the reaction. Examination of the benzene solution next day by T.L.C. (silica gel/chloroform/methanol) revealed a single new compound with a higher R.F. than that of the hydroxyacetate (28). It was the only detectable component of the reaction mixture. The reaction solution was worked up by washing with saturated aqueous $NaHCO_3$ solution, and water, and drying over Na_2SO_4. Concentration of the benzene solution *in vacuo* yielded a yellow oil (400 mgms). This was identified as the diacetate (34) (yield 66%, based on maximum theoretical yield of diacetate (600 mgms)).

P.M.R. ($CDCl_3$): δ 5.3 (t, 2H, C$\underline{H}$=C), δ 4.48 (d, 2H, CH—C$\underline{H}_2$—OAc), δ 4.35 (s, 2H, C—C$\underline{H}_2$—OAc), δ 2.1 (s, 4H, C$\underline{H}_2$—C=C), δ 2.0 (s, 3H, C$\underline{H}_3$—CO—O), δ 1.98 (s, 3H, C$\underline{H}_3$—CO—O), δ 1.70 (s, 3H, C$\underline{H}_3$—C=C), δ 1.64 (s, 3H, C$\underline{H}_3$—C=C).

I.R.: ($CHCl_3$, 0.5 mms) 1735 cm^{-1}, s, (OAc); 1260 cm^{-1}, s, (acetate C—O stretch).

Mass spectrum:

A) Chemical ionization with ammonia - 274 (M + 2 + 18, 3%), 273 (M + 1 + 18, 16%), 272 (M + 18, 100%), 69 (94%).

B) Electron bombardment - 195.1373 ($C_{12}H_{19}O_2$, M - 59, 2%), 150.1043 ($C_{10}H_{14}O$, 23%), 149.0239 ($C_8H_5O_3$, 42%), 135.0809 ($C_{10}H_{15}O$, 17%), 134.1093 ($C_{10}H_{14}O$, 85%), 127.0755 ($C_7H_{11}O_2$, 21%), 126 ($C_7H_{10}O_2$, 18%), 119.0860 ($C_9H_{11}O$, 57%), 93.0701 (C_7H_9O, 33%), 92.0621 (C_7H_8, 12%), 91.0546 (C_7H_7O, 21%), 85.0648 (C_5H_9O, 38%), 84.0576 (C_5H_8O, 100%), 79.0550 (C_6H_7, 19%), 80.0628 (C_5H_8, 40%), 55.0564 (C_4H_7, 23%).

Preparation of 10-tosyloxygeranylacetate

A solution of 10-hydroxy geranyl acetate (28) (500 mgms, 2.36 mmoles) in dry diethyl ether was prepared. The solution was cooled to 0°C and ^nBuLi (1.2 mls of 2.0 molar in n-hexane, 2.4 mmoles, 154 mgms) was slowly added. After 5 minutes, a solution of p-toluenesulphonyl

chloride (474 mgms, 2.5 mmoles) in ether was added. The mixture was stirred overnight with gradual warming to ambient temperature. Investigation of the reaction mixture next day indicated complete reaction. No 10-hydroxygeranyl acetate could be detected (T.L.C. silica gel/chloroform/methanol) but two new compounds were observed. These were at a higher R.f. value than the starting material. The reaction mixture was worked up by hydrolysis with aqueous pH 7 buffer and drying with Na_2SO_4. Concentration *in vacuo* gave a yellow oil. This oil was chromatographed on silica gel (Woelm, Grade II, special for dry column chromatography) using chloroform as solvent. Two fractions were collected, the one at high R.f. being a new compound, 10-chlorogeranyl acetate (45) the lower R.f. compound being the previously isolated 10-acetoxygeranyl acetate (44). The latter was identified by comparison of its spectral data with an authentic sample. 10-Chlorogeranyl acetate (45) was identified by the following spectral evidence.

P.M.R. ($CDCl_3$): δ 5.3 (t, 2H, $C\underline{H}=C$), δ 4.49 (d, 2H, $C\underline{H}_2-OAc$), δ 3.92 (s, 2H, $=C-C\underline{H}_2-Cl$), δ 2.1 (m, 4H, $C\underline{H}_2-C=$), δ 2.0 (s, 3H, $C\underline{H}_3-CO-O$), δ 1.71 (s, 6H, $C\underline{H}_3-C=C$).

I.R. (solution $CHCl_3$, 0.5 mms): 1730 cm^{-1} (s, C=O), 1280-1250 cm^{-1} (b, CH_2-Cl wag and acetate C—O stretch).

Mass spectrum:

A) Chemical ionization with ammonia - 250 (M + 2 + 18, 32%), 248 (M + 18, 100%), 230 (18%), 214 (16%), 212 (12%), 208 (3%), 206 (8%), 190 (1%), 188 (3%).

B) Electron bombardment - 230 (M, absent), 229 (M - 1, $C_{12}H_{18}O_2Cl$, 0.36%), 195 ($C_{12}H_{19}O_2$, 8%), 188 ($C_{10}H_{17}OCl$, 1%), 173 (12%), 166 (44%), 165 (21%), 155 ($C_{10}H_3O_2$, 19%), 149 (18%), 135 ($C_{10}H_{15}$, 73%), 134 ($C_{10}H_{14}$, 23%), 127 ($C_7H_{11}O_2$, 13%), 121 (C_9H_{13}, 31%), 119 (C_9H_{11}, 26%), 107 (C_8H_{11}, 30%), 105 (C_8H_9, 14%), 105 (7%), 103 (C_5H_8Cl, 22%), 101 ($C_6H_{13}O$, 32%), 94 (C_7H_{10}, 12%), 93 (100%), 91 (C_7H_7, 34%), 85 (C_5H_9O, 27%), 84 (C_5H_8O, 25%), 81 (C_6H_9, 35%), 80 (C_6H_8, 26%), 79 (C_6H_7, 21%), 77 (C_6H_5, 13%).

Preparation of N,N-diethylmethylsulphonamide

Diethylamine (60 mls, 42.6 gms, 0.58 moles) was slowly added over a period of two hours to a solution of methanesulphonyl chloride (30 mls, 44.4 gms, 0.39 moles) in CH_2Cl_2 at -40°C, while stirring. The mixture was warmed up to ambient temperature and then stirred for 24 hours. The reaction mixture was worked up by washing with saturated aqueous $NaHCO_3$ solution, pH 4 buffer, water and drying with magnesium sulphate. Concentration of the methylene chloride solution *in vacuo* yielded the crude sulphonamide. The latter was further purified by

distillation *in vacuo*. The fraction boiling at 92°/5 mms Hg was collected. This was pure N,N-diethylmethyl sulphonamide. Yield 23.5 gms (40%).

P.M.R. ($CDCl_3$): δ 3.22 (q, 4H, J = 7 Hz, $SO_2-\underline{CH}_2-CH_3$), δ 2.71 (s, 3H, $\underline{CH}_3-SO_2$), δ 1.19 (t, 6H, J = 7, $SO_2-CH_2-\underline{CH}_3$).

Preparation of "magic mesyl" (N,N,N-methyldiethylmethyl sulphonate).

N,N-diethylmethylsulphonamide (10 gms, 0.066 moles) was dissolved in methyl fluorosulphonate (20 mls, 28.2 gms, 0.24 moles) and heated to 50°C under dry N_2 for 3 days. The reaction mixture was diluted with methylene chloride (50 mls) and cooled to -78°C, whereupon the "magic mesyl" crystallized out. The crystalline ppt was filtered off under N_2 in a glove box and washed with dry CH_2Cl_2, and dried by suction. Yield of pure magic mesyl 9.46 gms (52%).

Preparation and solvolysis of 10-mesyloxygeranyl acetate

10-Hydroxygeranyl acetate (500 mgms, 2.36 mmoles) was dissolved in dry acetonitrile (50 mls) containing dry pyridine (0.30 mls, 0.29 gms, 3.41 mmoles). The solution was cooled to -45°C (F.P. of acetonitrile) and "magic mesyl" (1 gm, 3.77 mmoles) in acetonitrile added (2 mls). The solution was stirred at -45°C for 30 minutes and then

warmed to -10°C. It was then diluted with aqueous saturated $NaHCO_3$ solution (50 mls) and stirred at -5°C for 12 hours. The reaction product was recovered by work up with ether and careful washing of the ether layer to remove acetonitrile. After drying with sodium sulphate the ether layer was concentrated *in vacuo* to give a yellow oil (400 gms). This oil was chromatographed (silica gel, Woelm, Grade II for dry column chromatography, 40 gms) using methylene chloride as solvent. Two fractions were isolated. The higher R.F. fraction was identified by G.L.C. (OV 225 10" x 4 mms, 110-220 @ 8° min) as 10-acetoxygeranyl acetate and by spectrometric comparison with an authentic sample. The lower R.F. fraction was a mixture of two compounds (ratio 60/40) by G.L.C. (OV 225, 10" x 4 mms, 110-220 @ 8° min). Spectrometric evidence identified these as the isomeric allylic alcohols (28) and (46).

P.M.R. ($CDCl_3$) (of mixture):

Component A, 60%, (46)

δ 5.3 (t, 1H, C=C$\underline{H}$), δ 4.8 (d, 2H, C=C$\underline{H_2}$), δ 4.5 (d, 2H, C$\underline{H_2}$—OAc), δ 3.9 (t, 1H, CH_2—C$\underline{H}$—OH), δ 3.1 (s, 1H, O$\underline{H}$), δ 2.1 (m, 4H, CH_2), δ 2.0 (s, 3H, C$\underline{H_3}$—CO—O), δ 1.68 (s, 6H, C$\underline{H_3}$—C=C).

Component B, 40%, (28)

δ 5.3 (t, 2H, C=CH), δ 4.5 (d, 2H, C$\underline{H_2}$—OAc), δ 3.2 (d, 2H, C$\underline{H_2}$—OH), δ 3.1 (s, 1H, O$\underline{H}$), δ 2.1 (m, 4H, C$\underline{H_2}$—C=C),

δ 2.0 (s, 3H, $C\underline{H}_3-CO-O$), δ 1.68 (s, 3H, $C\underline{H}_3-C=C$), δ 1.60 (s, 3H, $C\underline{H}_3-C=C$).

Mass spectrum:

A) Chemical ionization with ammonia: 442 (2M + 18, 8%), 230 (M + 18, 100%), 214 (6%).

B) electron bombardment: 223 ($C_{12}H_{13}O_3$, M + 1, 0.56%), 152 ($C_{10}H_{16}O$, 5%), 150 ($C_{10}H_{14}O$, 30%), 149 ($C_8H_5O_3$, 24%), 135 (C_9H_{11}, 11%), 134 ($C_{10}H_{14}O$, 21%), 121 ($C_9H_{13}O$, 18%), 119 ($C_9H_{11}O$, 18%), 107 ($C_8H_{11}O$, 13%), 94 (C_7H_{10}, 12%), 93 (C_7H_9, 24%), 91 (C_7H_7, 12%), 85 (C_5H_9O, 24%), 84 (C_5H_8O, 100%), 81 (C_6H_9, 81%), 79 (C_6H_7, 20%), 71 (C_4H_7O, 22%), 69 (C_5H_9, 20%), 68 (C_5H_8, 47%), 67 (C_5H_7, 57%).

Preparation of Geranyl-p-anisate (50)

A solution of anisoyl chloride (50 gms, 0.34 moles) in benzene (100 mls) was slowly added to a solution of geraniol (53 mls, 46.2 gms, 0.3 moles) in benzene (500 mls) and triethylamine (40 gms, 55 mls, 0.4 moles) at 0°C. Throughout the addition the mixture was stirred and the temperature maintained at 0°C. After addition was complete the mixture was allowed to warm to room temperature and stirred for a further two hours. The reaction mixture was worked up by hydrolysis with pH 9 buffer and washing with water. After drying over Na_2SO_4 the benzene was concentrated *in vacuo* to give pure geranyl-p-anisate,

83.7 gms (97.5%).

P.M.R. ($CDCl_3$): δ 7.9 (d, 2H, J = 4, Ar—H), δ 6.8 (d, 2H, J = 4, Ar—H), δ 5.4 (t, 1H, C=C<u>H</u>), δ 5.0 (m, 1H, C=C<u>H</u>), δ 4.7 (d, 2H, $C\underline{H}_2$—O—CO), δ 3.72 (s, 3H, Ar—O-$C\underline{H}_3$), δ 2.04 (s, 4H, $C\underline{H}_2$—C=C), δ 1.73 (s, 3H, $C\underline{H}_3$—C=C), δ 1.62 (s, 3H, $C\underline{H}_3$—C=C), δ 1.54 (s, 3H, $C\underline{H}_3$—C=C).

I.R. ($CHCl_3$, 0.5 mms): 1708 (>C=O), ester, 1605 (benzene ring), 840 (m, O—Ar—H).

Mass spectrum:

A) Chemical ionization with ammonia; 323 (M + 35, 8%), 306 (M + 18, 100%), 290 (M + 2, 11%), 225 (10%).

B) electron bombardment; 152 ($C_8H_8O_3$, 44%), 149 ($C_8H_5O_3$, 17%), 136 ($C_{10}H_{16}$, 18%), 135 ($C_8H_7O_2$, 100%), 121 (C_9H_{13}, 13%), 93 (C_7H_9, 34%), 92 (C_7H_8, 7%), 80 (C_6H_8, 14%), 77 (C_6H_5, 22%).

<u>Selenium Dioxide Oxidation of Geranyl-p-anisate</u> (<u>50</u>)

A solution of selenium dioxide (28 gms, 0.25 moles) in hot 95% ethanol (250 mls) was slowly added to a stirred refluxing solution of geranyl p-anisate (<u>50</u>) (74.9 gms, 0.26 moles) in 95% ethanol (250 mls) at such a rate as to keep the mixture gently refluxing. After the addition was complete, reflux was maintained for 2 hours. The solution was cooled and decanted from the metallic deposit

of selenium, filtered and concentrated *in vacuo*. The residue, a dark yellow oil, was dissolved in ether (250 mls) and washed successively with 10% aqueous Na_2CO_3, water and saturated NaCl solution. After drying with Na_2SO_4 the ether was removed and the crude reaction product recovered. This was a complex mixture (>10 components). It was chromatographed by dry column chromatography (Woelm Silica Gel - Grade II for dry column) using chloroform as eluant. The aldehyde was easily located on the column by its strong quenching of the 254 nm fluorescence of the indicator. This product was rechromatographed by the same technique to give the pure aldehyde (50) 23.4 gms (30%).

P.M.R. ($CDCl_3$): δ 9.3 (s, 1H, R—C(=O)—H), δ 7.9 (d, 2H, Ar—H), δ 6.85 (d, 2H, Ar—H), δ 6.35 (t, 1H, C=CH), δ 5.45 (t, 1H, C=CH), δ 4.71 (d, 2H, CH_2—C(=O)—O), δ 3.8 (s, 3H, ϕ—OCH_3), δ 2.3 (m, 4H, CH_2—C=C), δ 1.77 (s, 3H, CH_3—C=C), δ 1.70 (s, 3H, CH_3—C=C).

I.R. ($CHCl_3$, 0.5 mms): 2820 and 2710 cm^{-1} (w, C(=O)—H stretch), 1705 cm^{-1} (—C(=O)—O), 1690 cm^{-1} (C=C—HC=O), 1605 (s, aromatic ring).

Mass spectrum:

A) Chemical ionization with ammonia; 320 (M + 18, 100%).

B) electron bombardment; 302 ($C_{18}H_{22}O_4$, 0.46%), 152 ($C_8H_8O_3$,

10%), 150 ($C_{10}H_{14}O$, 24%), 135 ($C_8H_7O_2$, 100%).

Zinc Borohydride reduction of Aldehyde (51)

A solution of aldehyde (51) (9 gms, 0.03 moles) in ether (100 mls) was prepared. This solution was cooled to 0°C and stirred while a solution of 0.2 M $Zn(BH_4)_2$ in ether (75 mls, 0.15 moles) was slowly added. When addition was complete the mixture was allowed to warm to room temperature and stirred for 1 hour. The ether solution was shaken in a sep. funnel with pH 7 buffer (100 mls) and the ether layer drawn off. This was washed with water (100 mls) and saturated aqueous NaCl solution (100 mls) and dried over Na_2SO_4. Removal of the ether by vacuum concentration gave an oil (5.9 gms, 64%).

P.M.R. ($CDCl_3$): δ 8.0 (d, 2H, Ar—H), δ 6.9 (d, 2H, Ar—H), δ 5.41 (m, 2H, =C—H), δ 4.8 (d, 2H, $-CH_2-O-\overset{O}{\overset{\|}{C}}-$), δ 3.96 (s, 2H, $-CH_2-OH$), δ 3.82 (s, 3H, $-O-CH_3$), δ 2.12 (s, broad, 4H, $CH_2-C=$), δ 1.76 (s, 3H, $CH_3-C=$), δ 1.67 (s, 3H, $CH_3-C=$).

I.R. ($CHCl_3$, 0.5 mms): 3450 cm^{-1} (m, broad, O—H), 1710 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-O$), 1605 cm^{-1} (s, aromatic ring).

Mass spectrum:

A) Chemical ionization with ammonia, 322 (M + 18, 100%), 305 (3.5%), 287 (18%), 279 (8%), 135 (37%).

B) electron bombardment, 304 (M , absent), 287 (M - 17, 8%), 219 (3%), 152 (18%), 135 (100%), 93 (18%), 92 (12%), 77 (21%), 68 (10%), 67 (18%), 41 (18%).

Preparation and solvolysis of 10-mesyloxy geranyl p-anisate (52)

10-hydroxy geranyl p-anisate (52) (500 mgms, 1.64 mmoles) was dissolved in dry methylene chloride (25 mls) and dry acetonitrile (25 mls) containing (0.20 mls, 0.19 gms, 2.3 mmoles) of dry pyridine added. The solution was cooled to -78°C and magic mesyl (0.65 gms, 2.5 mmoles) in acetonitrile (2 mls) was added. The solution was stirred at -78°C for 30 minutes and then warmed up to -10°C. It was then diluted with aqueous saturated $NaHCO_3$ solution (50 mls) and extracted with methylene chloride (3 x 20 mls). The combined extracts were washed once with water and dried over Na_2SO_4. Evaporation of the methylene chloride *in vacuo* gave the crude mesylate.

P.M.R. ($CDCl_3$): δ 7.98 (d, 2H, Ar—H, δ 6.85 (d, 2H, Ar—H), δ 5.4 (m, ~2H, =C—H), δ 4.7 (d, 2H, CH_2—O—C=O), δ 4.48 (s, ~1H, CH_2—O—Mes), δ 3.8 (s, 3H, ϕ—O—CH_3), δ 2.9 (d, 3H, CH_3—O—SO_2—), δ 2.2 (m, ~4H, CH_2—C=), δ 1.77-1.70 (m, ~6H, CH_3—C=).

I.R. ($CHCl_3$, 0.5 mms): 1710 cm^{-1} (s, $-\overset{O}{\overset{\|}{C}}-O-$), 1605 cm^{-1} (s, aromatic ring), 1190 cm^{-1} (m, $CH_3-\overset{O}{\overset{\|}{\underset{O}{\underset{\|}{S}}}}-O-$?).

Solvolysis studies were conducted by dissolving the crude mesylate in hexamethylphosphoramide (10 mls). It was found that solvolysis was essentially complete in 12 hours at room temperature. Work up was by dilution with water and extraction with methylene chloride. The only identifiable product of this reaction was the alcohol (52).

When perdeuteromethanol (2 mls) was added to the hexamethylphosphoramide (10 mls) the products isolated *via* the previous procedure were the perdeuteromethylethers (55) and (50).

P.M.R. ($CDCl_3$): δ 8.0 (d, 2H, Ar—H), δ 6.9 (d, 2H, Ar—H), δ 5.45 (m, ≃3H, H—C=), δ 4.9 (m, ≃1H, CH_2=C), δ 4.8 (d, 2H, CH_2—C(=O)—), δ 4.4 (t, ≃0.5H, CH_2—C—O=H), δ 3.82 (s, 3H, OCH_3), δ 2.2 (s, 4H, CH_2—C=), δ 1.75 (s, 3H, CH_3—C=), δ 1.65 (s, 3H, CH_3—C=).

D.M.R. (CCl_4): δ 3.74 (s, CD_3—O).

I.R. ($CHCl_3$, 0.5 mms): 1710 cm^{-1} (s, -C(=O)—O—), 1605 cm^{-1} (s, aromatic ring), 1270 cm^{-1} (s), 1110 cm^{-1} (C—O, stretch).

Mass spectrum:

A) Chemical ionization with ammonia, 339 (M + 18, 100%), 170 (27%).

B) Electron bombardment, 321 (M, absent), 288 ($C_{18}H_{18}O_3D_3$, 1%), 220 ($C_{13}H_{10}O_3D_3$, .4%), 169 ($C_{11}H_{15}OD_3$, 3%), 152 ($C_8H_8O_3$, 17%), 136 ($C_{10}H_{16}$, 5%), 136 ($C_8H_8O_2$, 8%),

135 ($C_8H_7O_2$, 100%), 134 ($C_{10}H_{14}$, 16%), 101 ($C_6H_7OD_3$, 22%), 93 (C_7H_9, 12%).

Preparation of 10-chlorogeranyl-p-anisate (57)

The crude mesylate (53) is suspended in saturated ammonium chloride for 1 hour at room temperature and the mixture extracted with methylene chloride. Concentration of the methylene chloride solution *in vacuo* gives extremely pure 10-chlorogeranyl-p-anisate (57) (quantitative yield).

P.M.R. ($CDCl_3$): δ 8.0 (d, 2H, Ar—H), δ 7.9 (d, 2H, Ar—H), δ 5.5 (m, 2H, H—C=), δ 4.8 (d, 2H, $C\underline{H}_2$—O—$\overset{O}{\overset{\|}{C}}$—), δ 3.98 (s, 2H, $C\underline{H}_2$—Cl), δ 3.82 (s, 3H, —O—$C\underline{H}_3$), δ 2.2 (m, 2H, $C\underline{H}_2$—C=), δ 1.75-δ 1.77 (s, 6H, $C\underline{H}_3$—C=).

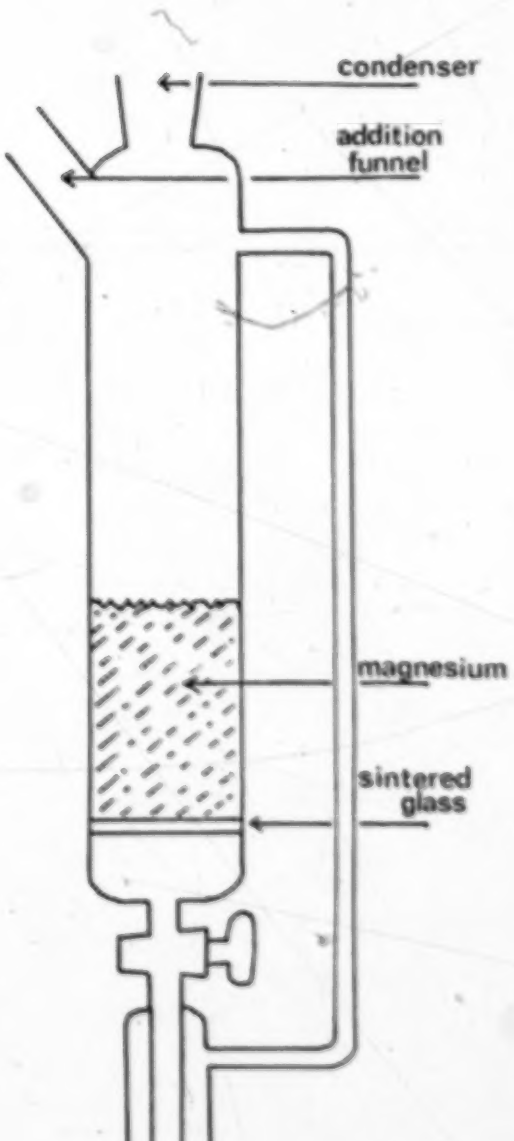
I.R. ($CHCl_3$, 0.5 mms): 1710 cm^{-1} (s, $\overset{O}{\overset{\|}{C}}$—O), 1609 cm^{-1} (s, aromatic ring), 700 cm^{-1} (s, C—Cl).

Mass spectrum:

A) Chemical ionization with ammonia, 343 (10%), 342 (33%), 341 (20%), 340 ($M^+ + 18$, 100%), 287 (10%).

B) Electron bombardment, 322 (M^+, absent), 287 ($C_{18}H_{23}O_3$, 10%), 219 (4%), 153 (40%), 136 (100%), 93 (30%).

Preparation and reaction of dimethylallyl magnesium bromide



Magnesium (special for Grignard reactions, 10 gms, 0.41 moles) was placed in the apparatus illustrated. The stopcock was closed and mercuric bromide (4 gms) in T.H.F. (50 mls) was introduced through the addition funnel. After 5 minutes the T.H.F. solution was drawn off. A flask containing dry diethyl ether was then attached to the bottom and refluxed for 10 minutes to remove any traces of mercuric bromide. This flask was replaced with another containing fresh dry ether and refluxing continued. Slowly over a period of 6 hours (14.9 gms, 0.1 moles) of 1-bromo-3-methyl-2-Butene dissolved in ether (20 mls) was added. The rate of addition was such as to keep the ether in the central chamber gently boiling. The formation of a white deposit collecting on the sintered glass

disc indicates magnesium bromide and the rate of addition is too fast. The Grignard reagent was collected in the lower flask.

To the Grignard solution was added 10-bromogeranyl-acetate (400 mgms, 1.46 mmoles) and the solution stirred under reflux for 8 hours. The reaction was worked up by pouring it into 100 mls of ice cold 3M $(NH_4)_2SO_4$ solution. This was extracted with ether, which after drying with Na_2SO_4 and concentration *in vacuo* gave a yellow oil (1.4 gms). This could be separated by prep. T.L.C. into three components. Two components were very non-polar indicating that they were hydrocarbons. The third component corresponded on T.L.C. with farnesol. It was identified as a farnesol isomer (59).

Mass spectrum: 222 (M, $C_{15}H_{26}O$, 1%), 204 (M - 18, 2%), 203 ($C_{15}H_{23}$, 1%), 107 (10%), 105 (8%), 93 (38%), 91 (35%), 8 (10%), 79 (15%), 77 (12%), 69 (100%), 67 (25%), 63 (14%).

P.M.R. ($CDCl_3$): δ 5.80 (d of d, 1H, J = 10, 16, $CH_2{=}C\underline{H}-$), δ 5.3 (t, 1H, J = 7, $=C\underline{H}\text{-}CH_2OH$), δ 5.0 (m, 1H, $=C\underline{H}-CH_2$), δ 4.8 (2d, 2H, J = 16, 10 $\underline{H}_2C{=}CH$), δ 4.0 (d, 2H, J = 7, $=CH-C\underline{H}_2OH$), δ 2.0 (m, 6H, $C\underline{H}_2-C=$), δ 1.7 (2s, 6H, $C\underline{H}_3-C=$), δ 1.0 (s, 6H, $C(CH_3)_2$).

I.R. ($CHCl_3$, 0.5 mms), 3600 cm^{-1} (m, OH), 1670 cm^{-1} (w, $HRC{=}CR_2$), 1640 cm^{-1} (w, $-CH{=}CH_2$), 910 cm^{-1} (m, $CH{=}CH_2$).

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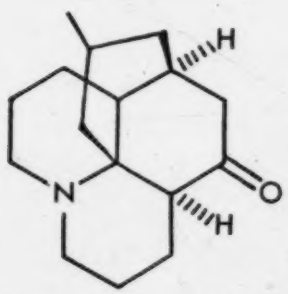
SOME TRANSFORMATIONS OF AN ANNOTININE DERIVATIVE.

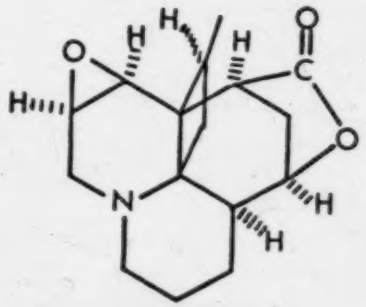
CHAPTER TWO

INTRODUCTION

The history of the Lycopodium alkaloids began in 1881 when Bödeker[60] investigated the chemical components of *L. complanatum*. He isolated an alkaloid whose description corresponds to one now known as lycopodine (70). In the intervening years much work has been done on the Genus Lycopodium and many more alkaloids have been isolated. The structure of lycopodine was not assigned until 1960, but the first lycopodium alkaloid structure was determined in 1957, when Wiesner characterized annotinine[61] (71). Both of these compounds have now been synthesized and their chemistry thoroughly investigated.



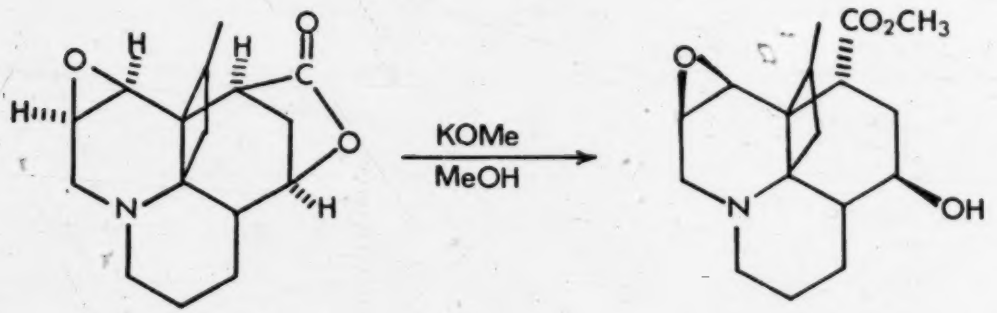
(70)



(71)

In these laboratories the chemical correlation of annotinine with lycopodine was undertaken by Braithwaite.[62] In the course of his studies he had occasion to prepare a derivative of annotinine - namely methyl epiannotinate

(72). This compound had been described previously by Marion *et al.*,[63] who prepared it by treating annotinine with metanolic potassium methoxide. Upon repeating their procedure Braithwaite detected a new compound in addition to methyl epiannotinate. This new derivate he called the mystery ester - or Ester "M", because of its unusual properties and elusive structure.

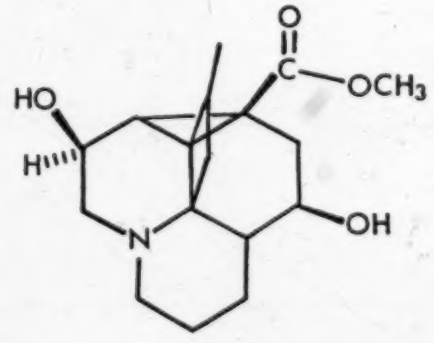


(71) (72)

Ester "M" was insoluble in most neutral solvents and very sparingly soluble in methanol. It was soluble in acids confirming its basic nature. The infrared spectrum showed a strong carbonyl band at 1719 cm^{-1}, and a broad hydroxy band at 3380 cm^{-1}. As the cleavage of a lactone by potassium methoxide gives a methyl ester and as ester "M" lacked the γ lactone absorption at 1780 cm^{-1} found in annotinine, this band must be due to an ester function. This fact was confirmed by P.M.R. which indicated a carbomethoxy function at δ 4.0. Lactone

cleavage produces an hydroxy group in addition to an ester group, and again this is confirmed by the formation of a diacetate of ester "M". Since annotinine has no hydroxy groups, the second hydroxy group must have come from cleavage of the epoxide moiety. The mass spectrum of ester "M" contained a parent peak at m/e 307. When compared to annotinine (M.W. 275) this clearly indicated the addition of one molecule of methanol. The base peak at m/e 248 corresponded to the loss of 59 m.u. and therefore a carbomethoxy group.

Gathering all these threads of evidence together one was inexorably drawn to the conclusion that ester "M" had the structure (73).



(73)

This structure accounted for the lower than usual I.R. value for the ester function. However 1719 cm^{-1}, is within keeping for a cyclopropyl ester. The stereochemistry shown was based strictly on the stereochemical relationships of the functional groups in annotinine. This

interesting structure and the stereochemistry was later confirmed by X-ray analysis of a derivative of ester "M".[64]

At this point a number of interesting questions arose. What was the precursor for ester "M" - was it annotinine or methyl epiannotinate? By what mechanism was ester "M" produced from either of these molecules? And what is the nature and stability of the cyclopropane ring in ester "M"?

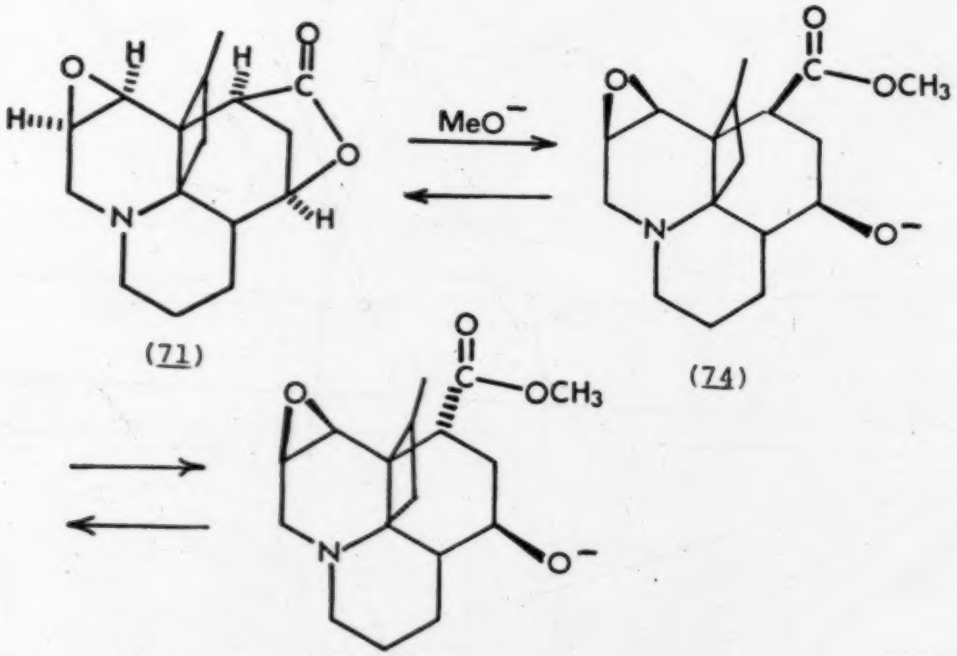
The first of these questions was further complicated by the fact that the formation of ester "M" was not reproducible. The exact combination of natural events required for its production remained elusive, despite various attempts to define them. One thing seemed clear however, that the potassium ion is essential, the reaction failing to occur with sodium or lithium methoxide.

The second question is relatively straightforward and there are many physical methods amenable to this problem.

The third question was certainly the most intriguing. There are no simple methods to determine the nature of the cyclopropane ring. One can observe its effects on neighboring functional groups and compare these to the standard values measured for other cyclopropane rings and one can perform certain standard reactions which are characteristic of cyclopropanes. This is what we intended to do.

DISCUSSION AND RESULTS

In order to distinguish between annotinine methyl epiannotinate as precursors for ester "M" it was decided to investigate thoroughly the reaction between annotinine and both sodium and potassium methoxide. One would expect such a reaction to proceed *via* a series of equilibria involving annotinine (71), methyl annotinate (74) and methyl epiannotinate (72).



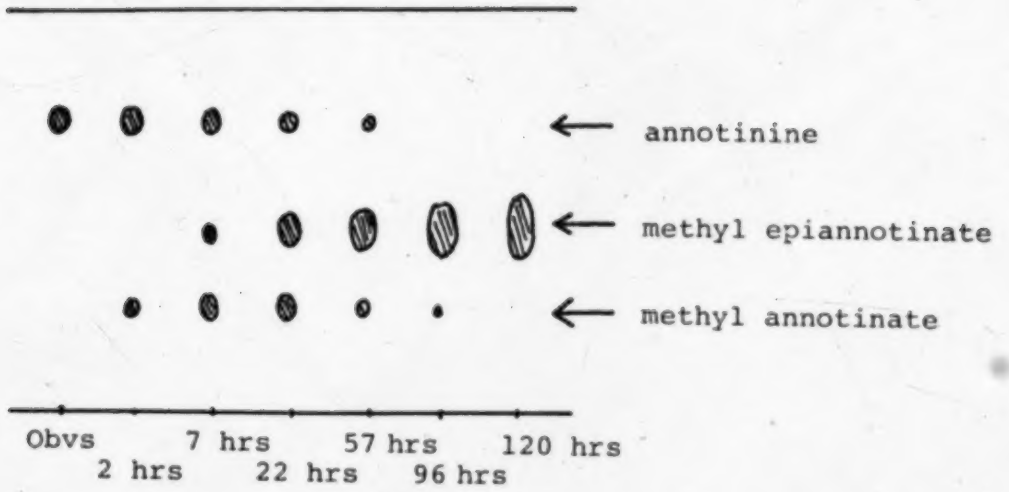
(71) (74)

(72)

To avoid the complication of concomitant formation of ester "M", sodium methoxide was used in initial experiments. It had been observed previously that little or no ester "M" is produced by sodium methoxide whereas potassium methoxide often gave high yields of ester "M".

The reaction of annotinine with 9 equivalents of sodium methoxide in methanol was carried out. The progress of this experiment was monitored by T.L.C. on silica gel. Aliquots were removed from the reaction mixture and the base present was neutralized by the addition of Amberlite IRC - 50. The latter is a weakly acidic carboxylic type ion exchange resin. When used in methanol as was the case here, there was no danger of the alkaloids being absorbed onto the resin. Thus the reaction solution after neutralization could be applied directly onto the T.L.C. plate.

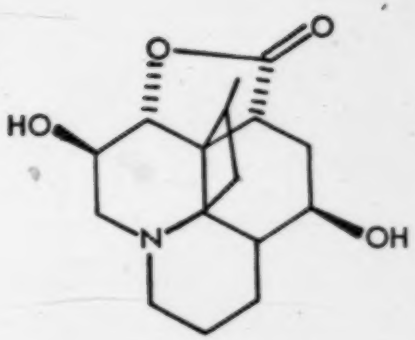
When studied by this method the reaction clearly proceeds *via* an intermediate. By comparison of R.f. values with authentic material this intermediate was identified as methyl annotinate. Over a period of 72 hours at room temperature the annotinine is gradually consumed, being replaced by methyl annotinate and ultimately by methyl epiannotinate.



The reaction can be speeded up by increasing the concentration of sodium methoxide. With the ratio of annotinine to sodium methoxide at 1:20, the reaction is essentially complete in 24 hours.

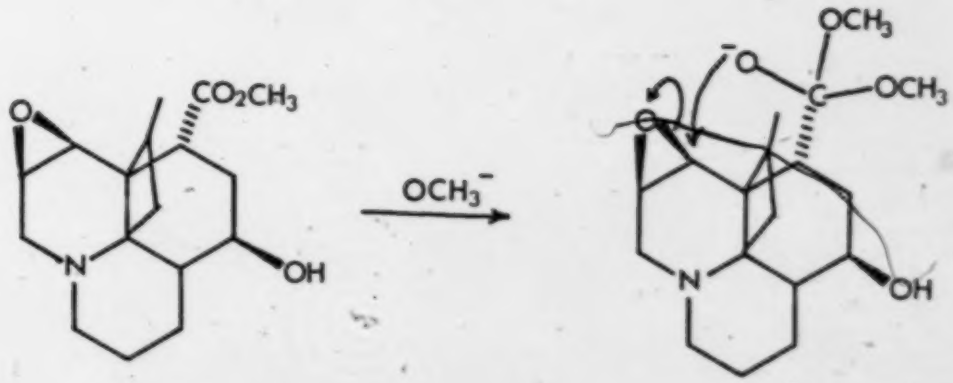
If the reaction solution was refluxed (B.P. of methanol 63°C) then the rate was speeded up but the product composition was altered with the appearance of a new component. This could be detected by T.L.C. as a new spot intermediate in R.f. value between annotinine and methyl epiannotinate. It was easily isolated by crystallizing the reaction product from a large volume of ether. The new product crystallized out before the methyl epiannotinate in hard colourless rhombs. It was quickly identified as

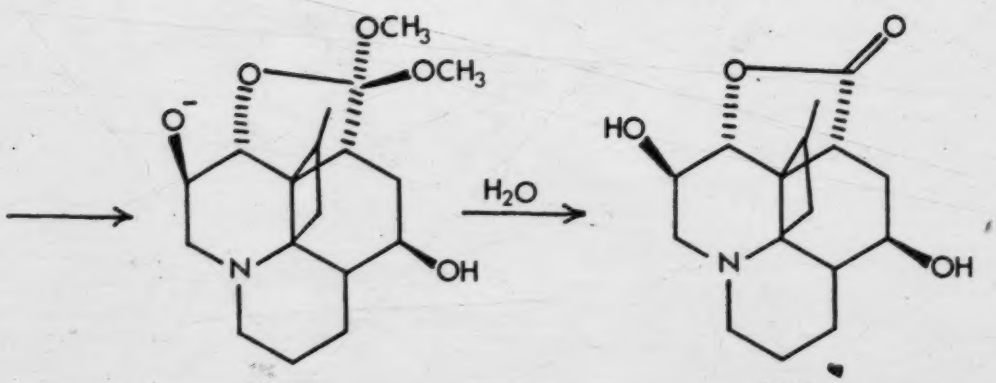
annotinine hydrate (75) by its M.P. and spectroscopic data.



(75)

The formation of this compound could be explained by attack of the ortho ester anion (75A) on the epoxide ring. Aqueous work up converts the ortho ester back into lactone giving annotinine hydrate (75).





(75A) (65)

With the nature of the reaction between annotinine and sodium methoxide thus examined we proceeded to look at the similar reaction with potassium methoxide.

Treatment of annotinine with 9 equivalents of potassium methoxide at room temperature gave a result entirely analogous with that for sodium methoxide. The reaction proceeded *via* the intermediacy of methyl annotinate and

gradually the sole component of the reaction mixture was methyl epiannotinate. There was no noticeable difference in the reaction rates. Refluxing the solution or heating in a pressurized system at 80°C did not cause formation of ester "M". It did result however in the formation of annotinine hydrate (75) as also was the case with sodium methoxide.

Thus at no point in the reaction between potassium methoxide and annotinine at room temperature in methanol could the formation of ester "M" be detected.

However in the course of working up these reactions an observation was made which cast some light on the formation of ester "M". Namely, reaction mixtures whose sole component was methyl epiannotinate, as determined by T.L.C. on silica gel, on evaporation under vacuum, followed by hydrolysis of the residue with water often gave large quantities of ester "M". Thus the production of ester "M" occurred when the methanol was evaporated without neutralizing the potassium methoxide.

The implication was clear - ester "M" is formed from methyl epiannotinate *via* a process which occurs during the evaporation of the solvent in the presence of potassium methoxide.

The first part of this hypothesis was easily checked - namely the involvement of methyl epiannotinate in the formation of ester "M".

Treatment of methyl epiannotinate with 9 equivalents of potassium methoxide in methanol followed by immediate evaporation under vacuum and hydrolylic work up gave a precipitate of ester "M". However if the above solution was simply diluted with a large excess of water and extracted with chloroform no ester "M" was formed and the majority of the methyl epiannotinate could be recovered. This important observation indicated that the formation of ester "M" occurred during work up of the reaction.

Therefore methyl epiannotinate was clearly the precursor for ester "M". Exactly how the conversion of the former into the latter occured, was not clear. There are two possibilities; firstly, that it is a concentration effect or secondly, that it is a heterogenous process which occurs in the solid state.

Some light was cast on this question by the observation that high water bath temperatures during rotary evaporation of the solvent produced better yields of ester "M". In fact if, after all the solvent was evaporated, the residue is heated in the water bath at 60°C for 5 minutes, an optimum yield of ester "M" was obtained. The yields under these conditions typically fall between 75% and 80%.

Although this does not clearly distinguish between the two possible explanations mentioned above it does lean favourably towards the heterogenous mechanism.

Further evidence on this matter can be evinced as follows. Ester "M" has a very high melting point (M.P. = 296° (dec.)) and it is very insoluble in all organic solvents. It is soluble in organic acids but this is a chemical reaction and therefore does not bear on the matter.

All of these facts indicate that ester "M" has a high lattice energy. However from a thermochemical point of view ester "M" has a higher free energy than methyl epiannotinate. It has a higher enthalpy due to the strain incurred by the formation of the new three membered ring and a lower entropy due to the reduced number of degrees of freedom in the molecule. Both of these factors combine to discourage the formation of ester "M". However it is formed and there must be an explanation.

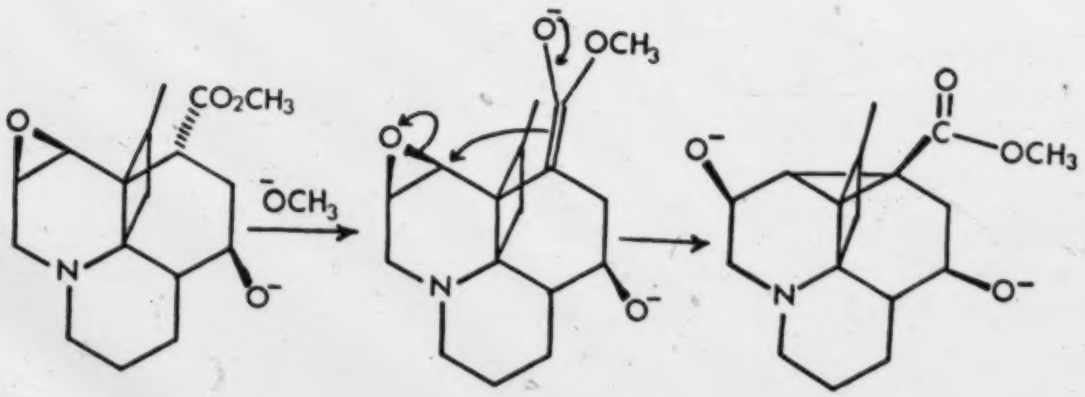
One such explanation is, that in the solid state the formation of ester "M" is permitted due to the offsetting effects of the lattice energy on the free energy. This agrees with the observation that ester "M" is not formed in solution. One can approach the relative values of the free energy and lattice energy by comparing published information on other compounds.

The strain energy of a cyclopropane ring is typically about 30 k.cals/mole. This value is observed in a wide variety of systems and therefore can be relied on. The lattice energy of a covalent compound is generally

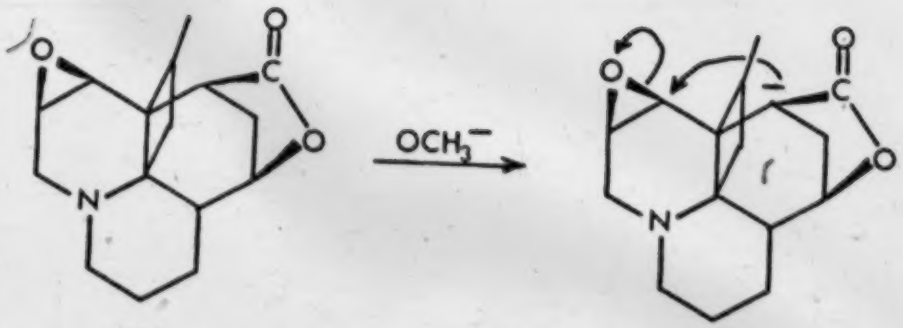
calculated to be equal to the heat of sublimation. Because many compounds do not sublime there is a shortage of suitable models for analysis. However the range of energies varies from a low of 12 kcals/mole for camphor to a high of 48 kcals/mole for stearamide. The difference here is 36 kcals, sufficient to ease the formation of a cyclopropane ring. In practise ester "M" compares with stearamide in that it is very difficult to sublime. The sublimation energy increases in proportion with the difficulty of sublimation. Of course the actual difference in energy between the lattice energy of methyl epiannotinate and ester "M" cannot be guessed at - it could concievably be of the order of 30 kcals/mole. The picture is complicated by the fact that what we should, in fact, be measuring is the difference between the lattice energy of the disodium salt of methyl epiannotinate and the disodium salt of ester "M". Furthermore, the latter salt is a weaker base than the former by three orders of magnitude.

All of these factors combine in the same direction - namely they favour the formation of ester "M". They have only qualitative importance - but this is adequate. One point not related to the above is that solid state reactions are rare because of slow diffusion rates. However this does not apply to intramolecular reactions and so need not be considered.

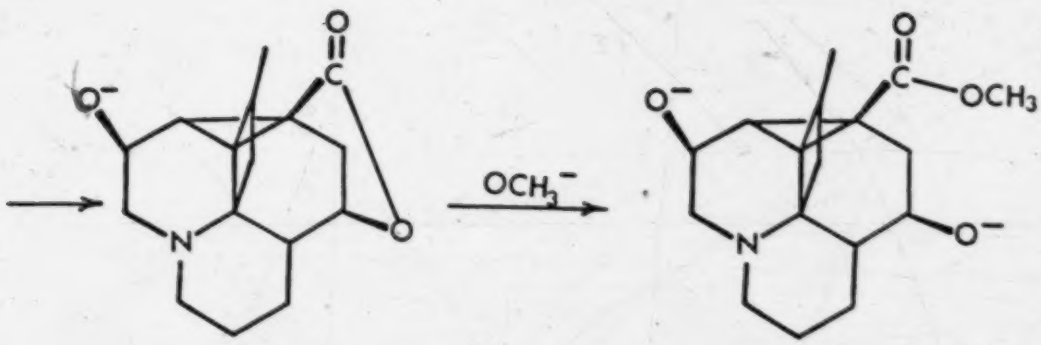
If one considers the mechanisms by which either annotinine or methyl epiannotinate can give rise to ester "M", it is obvious that the favoured precursor must be methyl epiannotinate.



(76) (77) (78)



(71) (79)



(80) (81)

Anion (77) shows normal resonance stabilization *via* the ester carbonyl group while the anion (79) formed from annotinine is anti-Bredt and therefore can receive no resonance stabilization. Such anions are very difficult to form and it is doubtful whether sodium methoxide is sufficiently basic for their generation.

Further proof that annotinine is not involved in the formation of ester "M" comes from the observation that a freshly prepared solution of annotinine and nine equivalents of potassium methoxide upon immediate evaporation at room temperature and treatment of the residue at 60°C, gives no detectable quantity of ester "M". Furthermore, treatment of annotinine with potassium tert-butoxide in tert-butanol followed by refluxing and evaporation of the solvent and heating of the residue does not give rise to ester "M". The use of potassium t-butoxide should prevent cleavage of the lactone function due to the steric hindrance encountered by such large molecules. However potassium t-butoxide is a stronger base than potassium methoxide and thus should not prevent

formation of the anion (79). The fact that no ester "M" was observed as product means that either the anion (79) does not give rise to ester "M" or that it was not formed under the reaction conditions. Either way the possibility of annotinine as a direct precursor for ester "M" need not be considered further.

There remained, however, one aspect of the mechanism for the conversion of methyl epiannotinate to ester "M", that was still unclear. This concerned the large effect that the nature of the cation had on the yield of ester "M". It had been observed that whereas potassium methoxide gave good yields of ester "M", that sodium methoxide gave little or none. It was decided therefore to investigate all the group I metals (except cesium and francium) in order to determine their ability to produce ester "M". If the observed effect was due to the differences in ionic radii between sodium and potassium then lithium should produce no ester "M" and rubidium should be equivalent to potassium. Such effects have been noted for many different reaction types[68] and when enhanced by large cations show the order $Li^+ < Na^+ < K^+ < Rb^+ \simeq R_4N^+$. Accordingly, the preparation of ester "M" was attempted under the standard conditions with each of the above cations and the following results were obtained.

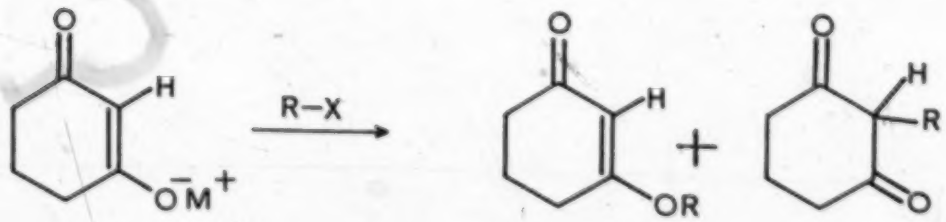
cation	yield of ester "M"
Li^+	0%
Na^+	<5%
K^+	81%
Rb^+	21%

All of these reactions were monitored by T.L.C. and all showed the conversion of annotinine to methyl epi-annotinate *via* methyl annotinate. The value obtained for the reaction with rubidium metal is possibly incorrect as great difficulty was encountered in handling this metal. In order to remove it from the container (a vial) it had to be melted (M.P. 39°C) and this combined with its extreme reactivity resulted in some deterioration. Even though it was manipulated throughout in a dry nitrogen atmosphere it became immediately coated with a white layer. It is also possible that the lower yield obtained with rubidium as opposed to potassium is significant. In this case it may be that the ionic radius of rubidium is too large and that of sodium is too small, when compared to that of potassium, which may have the optimum size for this reaction.

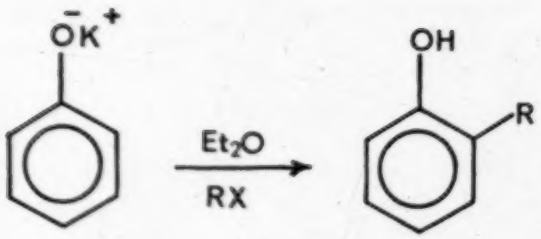
Furthermore this observation is in keeping with the previously described solid state mechanism for the preparation of ester "M". Variations in ionic radius would be reflected in different values for the lattice

energy for the di-metal salt of ester "M". And if this lattice energy is regarded as the driving force for the reaction such variations should show up in the yield obtained.

Of course similar effects on reaction yields due to ionic radius have been observed in homogenous reactions in various solvents.[66] These effects can be explained on the basis of electronegativity. It was observed that in the case of ambident anions that more electropositive metals caused more o-alkylation, e.g.,



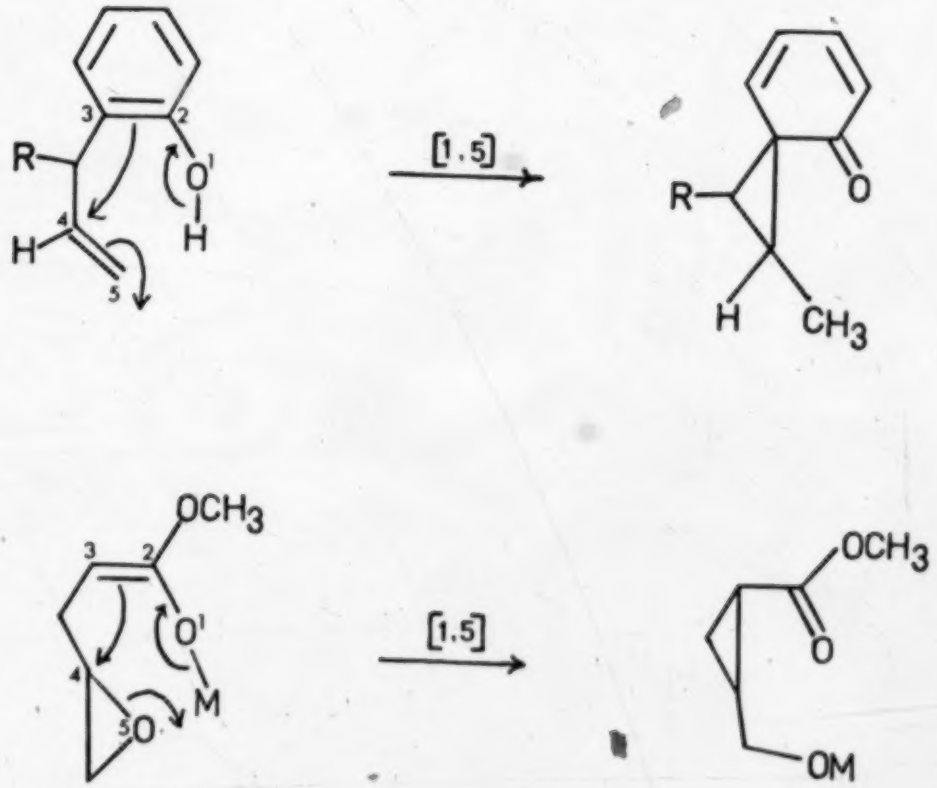
However, this does not apply to the case of ester "M" as the anion (77) involved is not ambident. Furthermore the formation of ester "M" is clearly a heterogenous process. It has been observed that in the case of ambident anions reacting under heterogenous conditions that C alkylation is exclusively obtained,[67] e.g.,



100%

This is explained by the oxygen being very strongly bound to the cation in the crystal lattice and thus unable to react *via* o-alkylation. This is only observed in ambident anions and of course those which normally give exclusively o-alkylation. Therefore there is little analogy between this case and that of ester "M" formation.

There is one final possibility that can account for the observed effect of the nature of the cation on the yield of ester "M". This arises out of the observation that the formation of ester "M" bears some analogy to the abnormal Claisen rearrangement.[70] The latter reaction can be regarded as a [1,5] sigmatropic shift of a hydrogen. The formation of ester "M" corresponds loosely to a [1,5] sigmatropic shift of a cation.



The equivalency of double bonds and three membered rings in sigmatropic rearrangements is well known.[42] There is no intention here to suggest that a cationotropic rearrangement such as that of ester "M" is indeed sigmatropic. Instead however it is of interest to compare the stereochemical requirements of a [1,5] sigmatropic rearrangement and apply these to the cationotropic

rearrangement of ester "M". First of all, sigmatropic rearrangements are concerted. Thus the hydrogen lost from the phenolic group in the abnormal Claisen is the hydrogen which adds to the terminus of the double bond. Secondly, the locus of this particular hydrogen atom does not move appreciably in the process.

If one fits these requirements to the formation of ester "M" some interesting analogies appear. It is only in the solid state that the cation is closely bound to oxygen anion (77). In solution the cation is rapidly transferred from one anion to the other. It is only in the solid state that the cation adopts a defined position with respect to the anion. This position must be one which allows the cation to transfer from the oxygen of the epoxide anion without appreciable movement. The distance between these two oxygens is approximately 4Å (as determined from molecular models). To occupy an intermediate position the cation would have to be large. Both potassium and rubidium with ionic diameters of 2.6 Å and 3 Å satisfy this requirement. The ionic diameters of lithium and sodium are 1.2 Å and 2 Å. .

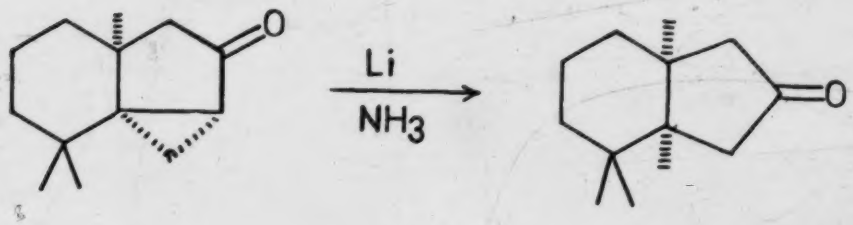
There is of course no way that this idealized picture can be verified. The stereochemical tests that confirm the sigmatropic nature of a rearrangement cannot be applied to the enolate anion or the epoxide ring. However it is useful to make the comparison as it gives some

insight into the nature of the reaction. Furthermore, the observation that in heterogenous alkylation reactions the cation is strongly bound to oxygen lends some weight to the above possibility. It is also possible that although the reaction is not concerted, the metal cation which becomes attached to the epoxide oxygen is from a neighbouring enolate ion in the lattice structure. Thus only two cations would be involved in the rearrangement and of course changes in the crystal structure due to the nature of the cation would cause dramatic effects as the inter-ionic distances varied.

Having thus dealt with the mechanistic aspects of the formation of ester "M" it was also of interest to provide some more structural information. It was felt that because of the unusual pentacyclic nature of ester "M" and the high potential energy associated with such a structure that ester "M" should participate in reactions which would cleave the cyclopropane ring. Such reactions could at the same time provide a chemical proof of the structure of ester "M". The ease with which these reactions proceeded might also give some indication of the strain present in the molecule. We thus set about to regenerate the tetracyclic skeleton of annotinine from the pentacyclic skeleton of ester "M".

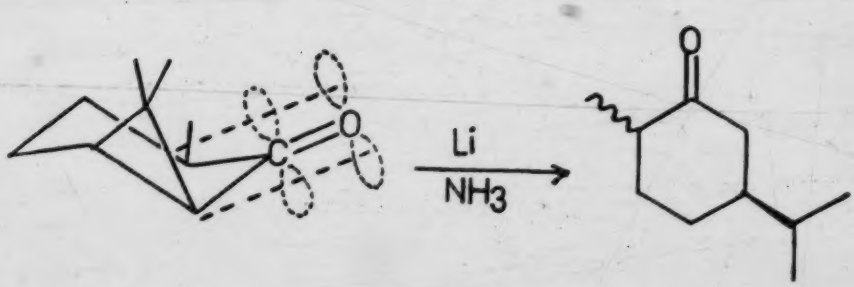
One such reaction which could be used to cleave cyclopropane ring was the reduction of cyclopropyl ketones

using lithium in ammonia. This reaction was pioneered by Norin, *et al.*,[69] and later studied by Dauben and co-workers.[70] They showed that cyclopropyl ketones are rapidly reduced by lithium in ammonia in high yields. Thus thujopsene (82) is quantitatively reduced to its methyl derivative (83).

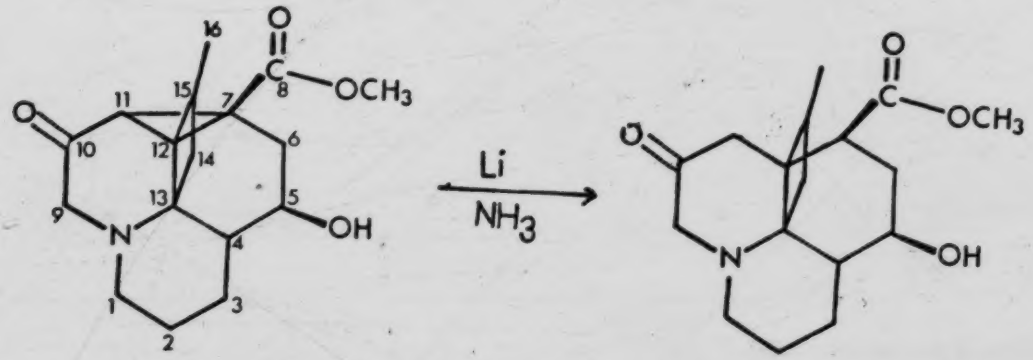


(82) (83)

What is more interesting however is that only that bond of the cyclopropyl ring which overlaps efficiently with the carbonyl group is cleaved in the reaction, e.g.,

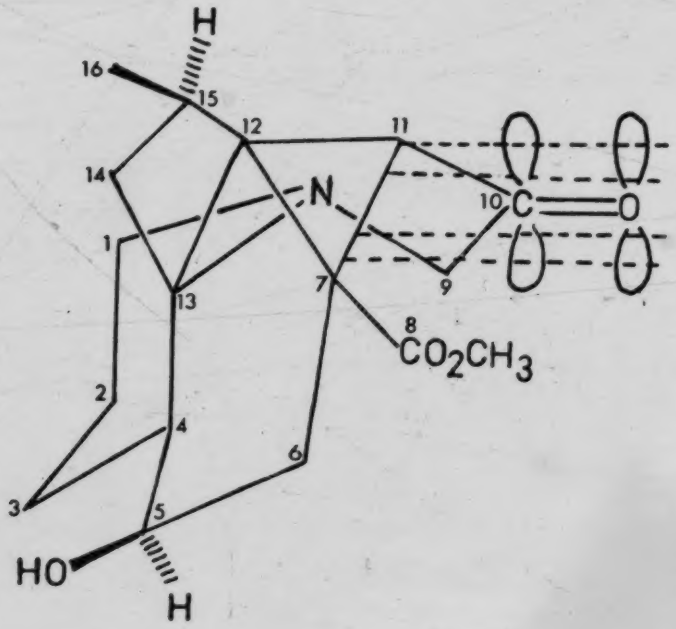


If this analogy is applied to the 10-ketone derivative of ester "M" (84) then the 7, 11 bond should be cleaved in the product (85), e.g.,

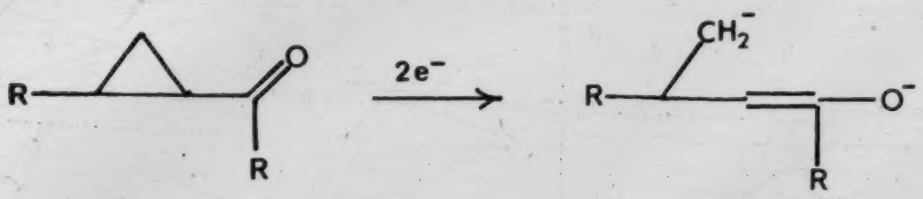


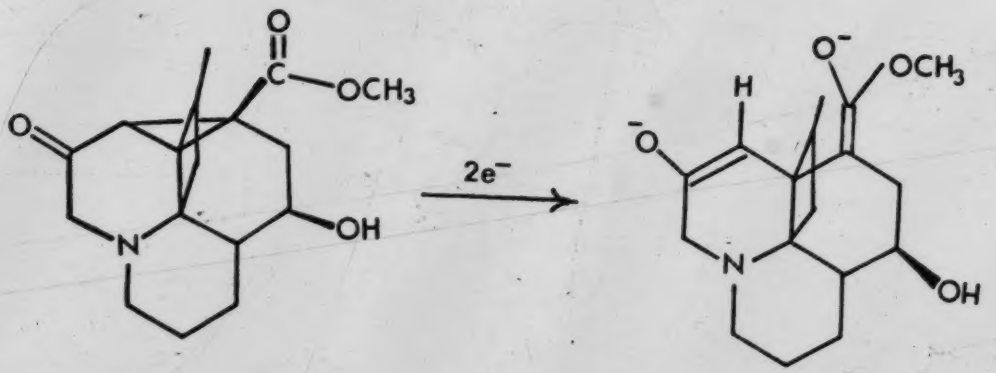
(84) (85)

It is obvious when one examines the models that only the 7, 11 and not 11, 12 bond overlaps efficiently with the π system of the carbonyl



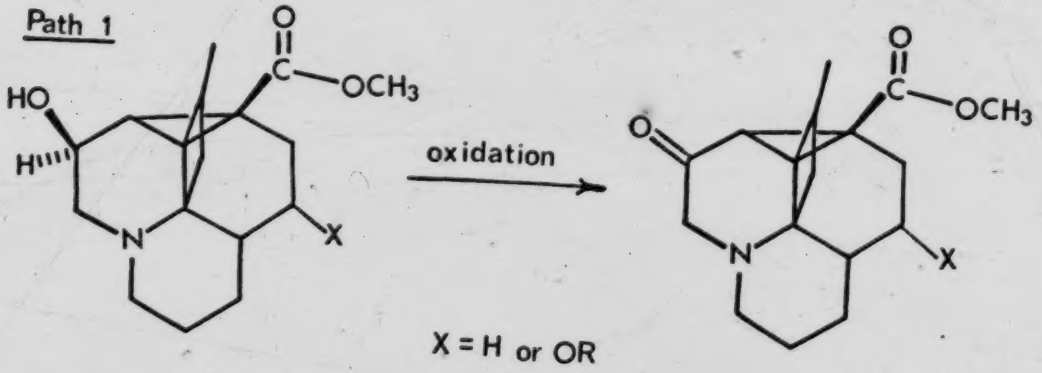
Furthermore, although the reduction of cyclopropyl ketones proceeds efficiently when there is no special stabilization of the intermediate carbanion, such a carbanion would be stabilized in the case of ester "M" and the reactivity should therefore be enhanced, e.g.,

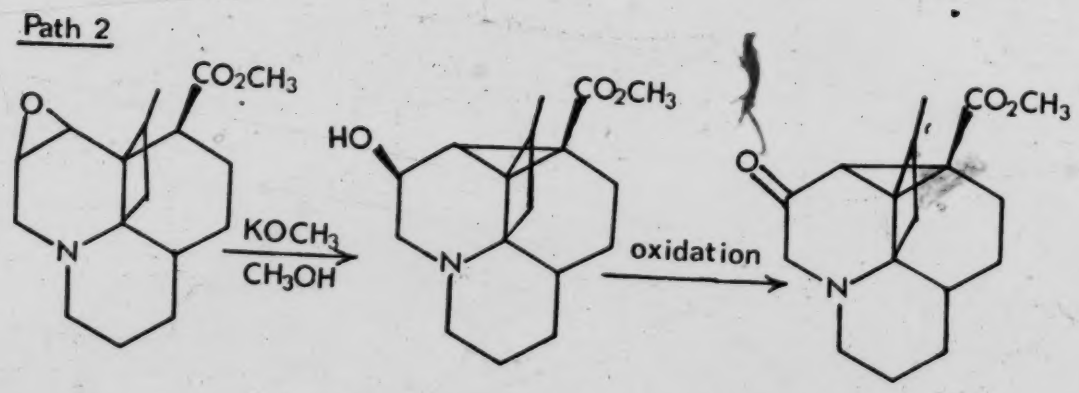




There are two approaches to the preparation of ester "M" with a ketone function at the 10 position. One can modify the ester "M" molecule in such a way that one of

the hydroxy groups (i.e., the one at C-10) is oxidized while the other is either blocked or removed. Alternatively one can prepare an analogue of ester "M" lacking a hydroxy group at C-5 from a suitably deoxy precursor such as methyl-5-deoxy-epiannotinate (86).



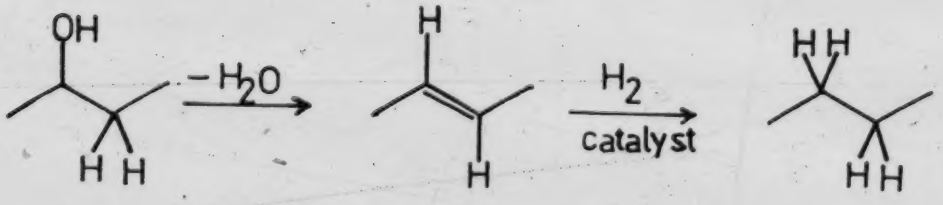


(86)

Because path (2) allowed an interesting test to be made concerning the generality of the ester "M" cyclization reaction it was investigated first. The ability of

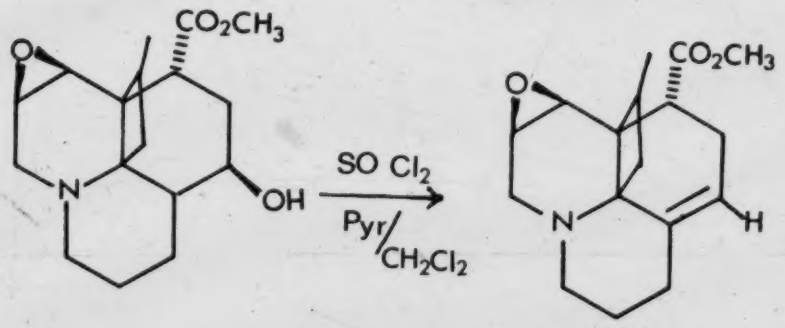
methyl-5-deoxy-epiannotinate (86) to react in an analogous fashion to methyl epiannotinate (82) should clearly demonstrate that the 5 hydroxy group is not involved in the formation of ester "M". However the result if negative should provide some useful information.

The simplest method of removing hydroxy groups is to eliminate them to form the olefin. The olefin can then be catalytically hydrogenated to give the deoxy hydrocarbon, e.g.,



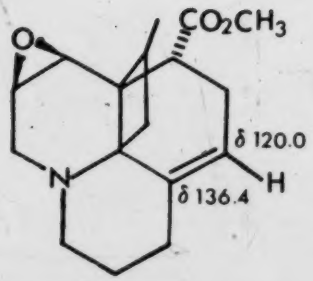
Accordingly methyl epiannotinate was treated with 1.5 equivalents of thionyl chloride in methylene chloride as solvent with 3 equivalents of pyridine added. The solution was stirred at room temperature for 20 minutes by which time T.L.C. analysis on silica gel indicated that the reaction was complete. Work up was by means of dilution with saturated sodium bicarbonate solution followed by overnight high vacuum treatment to remove

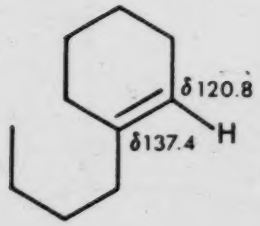
pyridine which gave a new compound identified as methyl anhydro-epiannotinate (87).



(87)

The P.M.R. of this compound shows a single olefinic proton as a triplet at δ 5.7. The appearance of only a single olefinic proton confirms that the double bond is indeed the Δ4(5) isomer. Further confirmation comes from the C.M.R. spectrum. This clearly indicates two olefinic carbons at δ 136 and δ 120. Carbon 4 which is fully substituted absorbs at δ 120. This compares favourably with values of δ 137.6 and δ 120.8 for carbons 1 and 2 of 1-butyl cyclohexene.



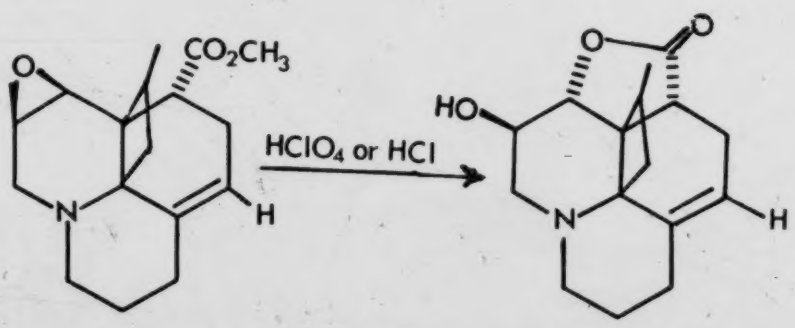


Of course the Δ 4(5) isomer is the expected product. Although both carbons 4 and 6 in (73) have hydrogen antiperiplanar to the hydroxy group at carbon-5 only elimination across the 4,5 bond can produce a trisubstituted isomer.

Treatment of (87) with hydrogen and platinum oxide at atmospheric pressure in ethanol as solvent was without effect. Similarly, 10% palladized charcoal and 5% rhodium on alumina was also without effect. Increasing the pressure of hydrogen to 40 P.S.I. and raising the temperature of the solvent to 70°C again had no effect. In all cases the starting material could be recovered intact. This result is not unusual. Trisubstituted double bonds are often difficult to hydrogenate and especially so when hindered which was the case here. In certain cases this problem can be overcome by the use of acid catalysts

such as perchloric acid or using acetic acid as solvent.[71] However in (87) the epoxide is sensitive to acid. For this reason the reaction using acetic acid as solvent was tried first.

Accordinly (87) was dissolved in acetic acid and hydrogenated at 40 p.s.i. using platinum oxide as catalyst. After 12 hours a small amount of new material was formed but the majority of the starting material was intact. Addition of 1 drop of perchloric acid to the reaction solution resulted in a complete conversion of the starting material to this new compound. The conversion could also be achieved by treating (87) with 5% aqueous HCl. This new compound was isolated by a combination of sublimation and chromatography and identified as lactone (88).



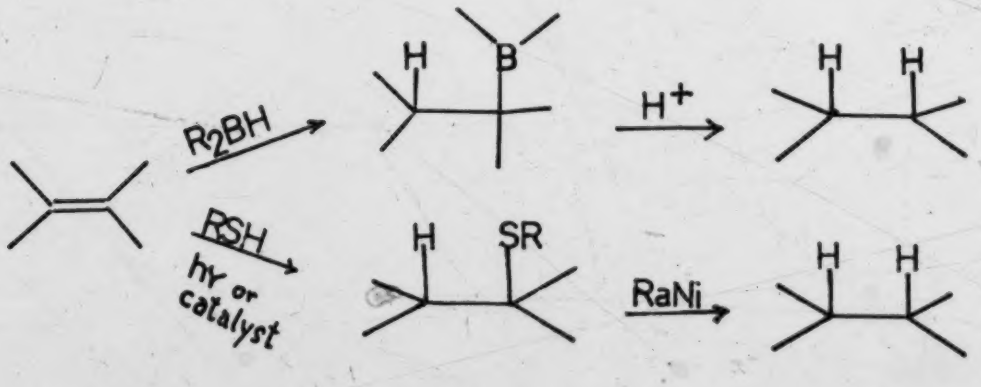
(87) (88)

The lactone (M.P. 155-158°C) showed a carbonyl absorption at 1785 cm^{-1} and a hydroxy group at 3420 cm^{-1}. The P.M.R. showed an olefinic proton at δ 5.6 and a

lactone proton at δ 5.5. There was no carbomethoxy group absorption. Mass spectral measurement gave a molecular weight of 275.

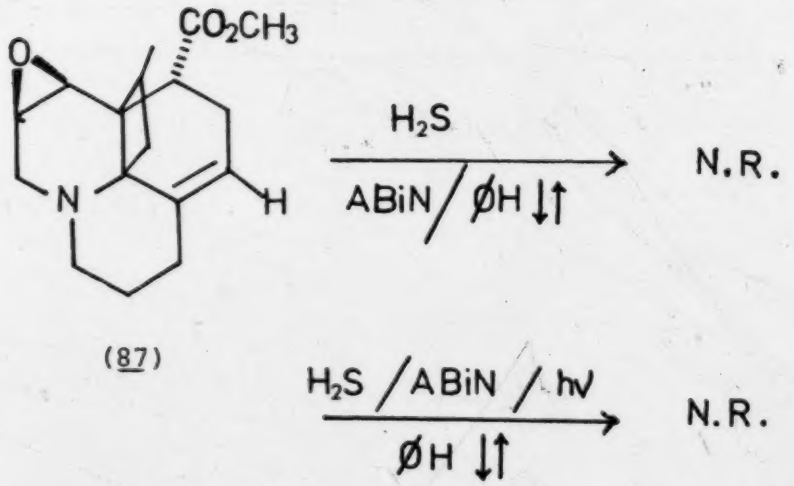
It was felt that the failure of the hydrogenation reaction to proceed, derived from the stereochemical difficulties of absorbing the hindered double bond of (77) to the surface of the catalyst. Therefore the reaction might proceed if a less heterogenous catalyst was used. One solution would be the use of homogenous catalysts such as triphenyl phosphine rhodium chloride.[72] However this catalyst and similar congeners are singularly ineffective against trisubstituted olefins.[73]

Nevertheless there are other reactions which involve addition to a double bond by homogenous processes. Two such examples are the addition of boranes[74] and thiols.[75] Furthermore both addition products can be converted to the hydrocarbon by simple processes.



The addition of boranes seemed unsuitable for our purposes as the protolysis in the second step was likely to destroy the epoxide in (87). The addition of thiols seemed in all respects excellent, as the first step is a free radical process and the second step is mild. The other functional groups in the molecule are stable to Raney Nickel.

A solution of (87) in benzene was saturated with hydrogen sulphide and a catalytic amount of azobisisobutyl nitrile added. The solution was refluxed for 24 hours, by which time no reaction had occurred. It was then irradiated with a low pressure mercury lamp for an additional 48 hours with continuous refluxing. No reaction occurred and the starting material was recovered intact.



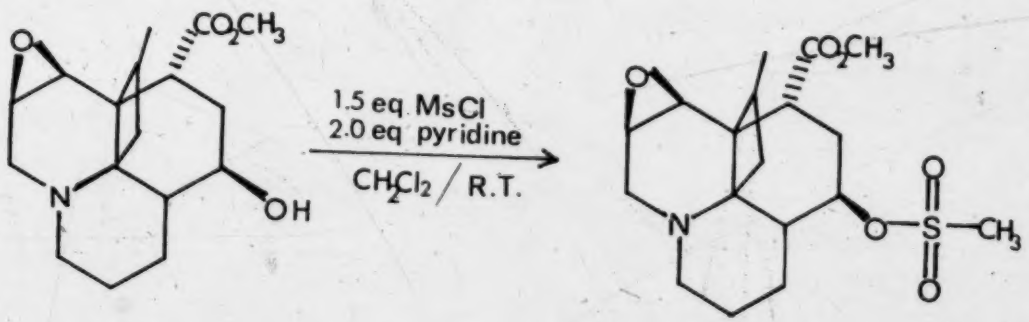
(87)

H_2S / ABiN / hν, ØH ↓↑ → N.R.

We abandoned any further procedures which involved the double bond and instead chose to remove the hydroxy group by means of a derivative with good leaving group properties.

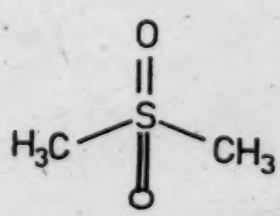
Such a leaving group is the mesylate function. There are many methods in the literature for removing mesylate groups using mild reducing agents such as cyanoborohydride.[76]

Treatment of methyl epiannotinate (72) with 1.5 equivalents of mesyl chloride and 2.0 equivalents of pyridine in methylene chloride gave as a sole product the mesylate (89). This mesylate was unexpectedly stable and could be chromatographed on silica gel without decomposition.



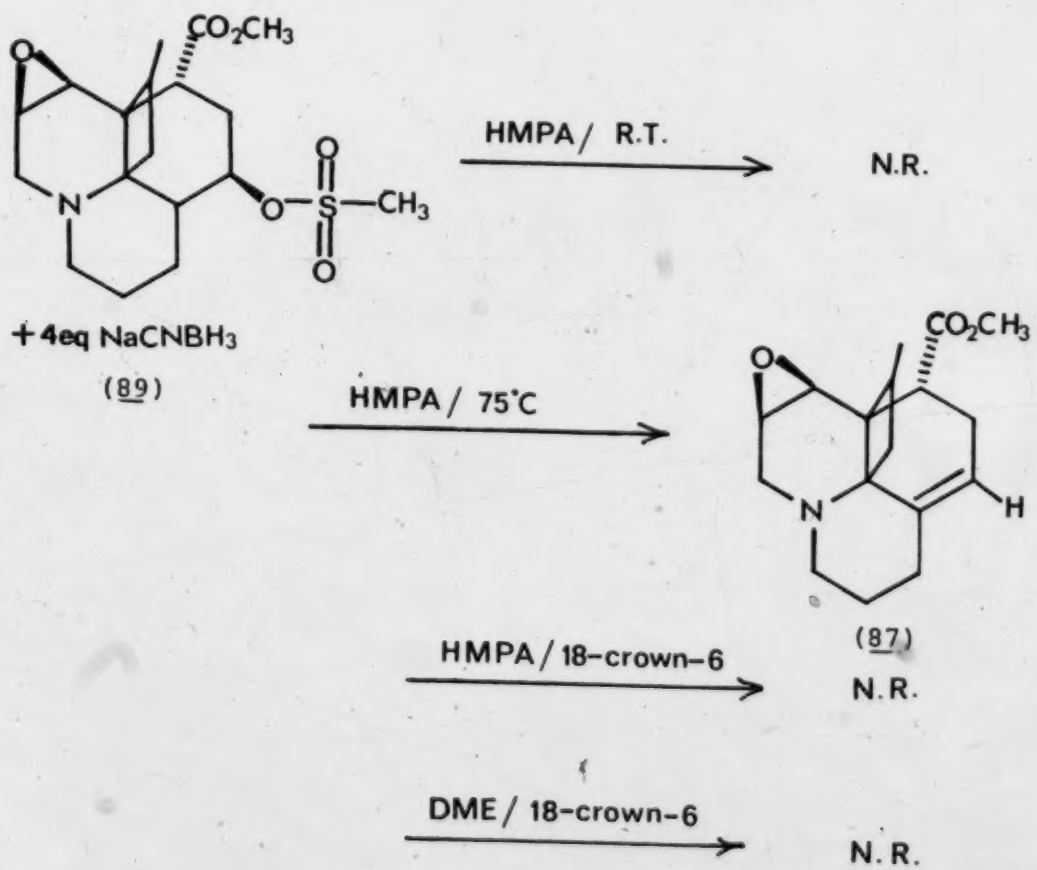
(72) (89)

The structure of (89) was amply confirmed by spectral evidence. C.M.R. showed the presence of 18 carbons, one of which, when compared to (84) was new. This signal occured at δ 38.6. Although no literature values for a mesylate could be found this compares favourably for values for sulphones[77], e.g.,



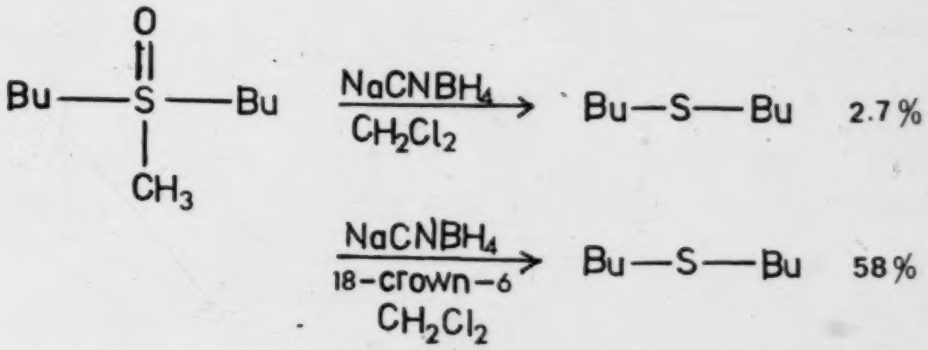
Similar corroboration came from P.M.R. The presence of a mesyl methyl group was indicated by a three proton singlet at δ 3.01. Furthermore the C-5 proton appears at δ 4.9 as a multiplet. When compared to the value for the proton in (87) this corresponds to a downfield shift of 1.1 ppm. This is in excellent agreement with similar shifts on going from an alcohol to an ester.

Reduction of the mesylate according to the method of Hutchins, *et al.*,[76] using sodium cyanoborohydride in hexamethylphosphoramide failed to take place.



The use of elevated reaction temperatures gave a small amount of olefin (87) but no reduction product. The olefin could easily be detected by the presence of the olefinic proton in the P.M.R.

The observation of Durst concerning the effect of crown ethers on cyanoborohydride reductions prompted us to try such a procedure.[77] Durst noticed that the reduction of alkoxy sulphonium salts to sulphides in methylene chloride was catalysed by 18-crown-6, e.g.,

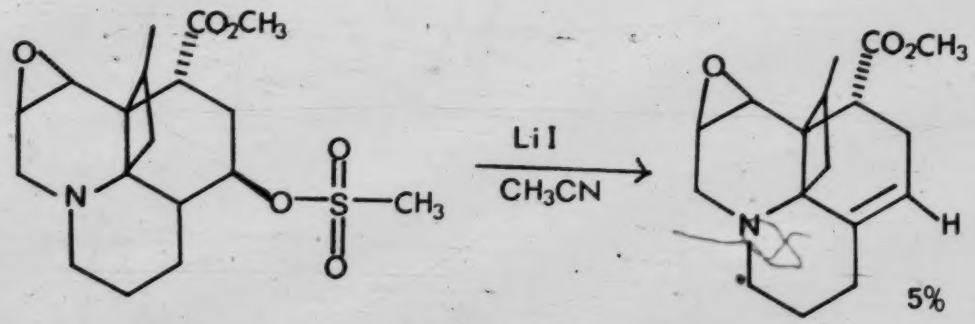


The crown ether catalyses the reaction by selectively complexing the sodium cation. This increases the net charge on the cyanoborohydride anion and thereby also its nucleophilicity. However this method did not aid the reduction of (89) by cyanoborohydride in our case. Attempts using 0.01 equivalents and 0.1 equivalents of 18-crown-6 were equally disappointing. The reaction was tried both in HMPA and DME and at room temperature

and 70°C. In all cases no reduction was observed. This could only have been due to the large degree of steric hindrance at carbon 5 in the mesylate of methyl epiannotinate.

To test this latter possibility another approach was undertaken to displace the mesyl group using one of the better nucleophiles - namely iodide ion. If anything was capable of displacing the mesyl group, this would surely do it.

Treatment of 1 equivalent of methyl epiannotinate with a large xs of lithium iodide in acetonitrile at room temperature for 24 hours resulted in the production of a small quantity of olefin but otherwise no iodides could be detected.



(89) (87)

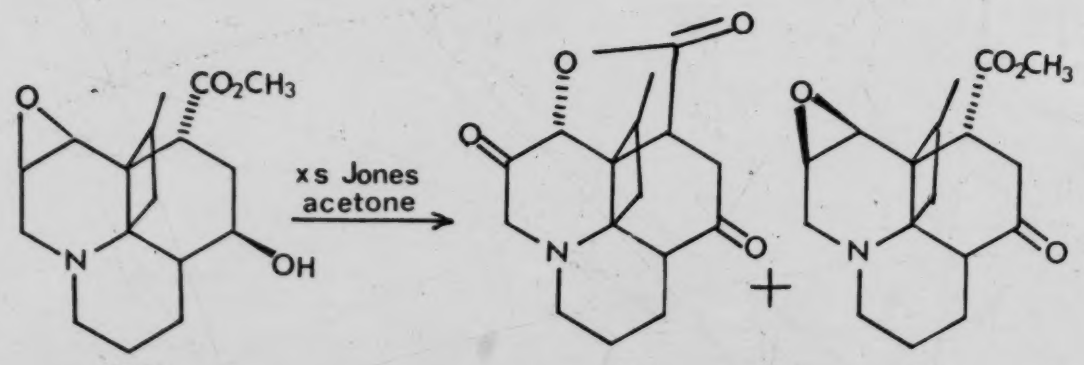
Refluxing the reaction solution (B.P. acetonitrile = 80°C) served only to increase the production of olefin. Throughout these experiments the reaction mixture was dark yellow - no doubt due to iodine by a side reaction.

Assuming that the iodine might be responsible for the production of olefin it was decided to use a modification developed by Corey to avoid the production of iodine.[78] This involved the addition of mercury to the reaction mixture. When the reaction is carried out in the presence of mercury the production of olefin is essentially stopped but no reaction is observed.

These experiments amply confirmed the premise that the C-5 position of methyl epiannotinate is extremely hindered. We abandoned any further attempt to remove the C-5 hydroxy group by means of nucleophilic displacement. This left quite a number of routes still open to us. Two of the most promising involved oxidation at C-5 to a ketone and removal of the ketone by any of the number of methods available for this purpose. Alternatively the hydroxy group could be blocked by a suitable reagent which was inert to strong base. Again there are quite a number of these, such as tetrahydropyranyl ethers, simple methyl ethers and certain silyl ethers such as t-butyl-dimethylsilyl ether.

Of these two possibilities the ketone route seemed the most promising. Conversion of the C-5 centre from SP3 to SP2 symmetry should result in a flattening of the ring at that site. This should ease the approach of any attacking reagent and relieve the problems encountered due to steric hindrance.

Accordingly a solution of methyl epiannotinate in acetone was treated with Jones' Reagent (8 M aqueous) until the orange colour was sustained. The reaction was stirred for a further five minutes and then quenched with isopropanol. The acetone solution was decanted from the polymeric chromium, diluted with an excess of methylene chloride and washed with saturated sodium bicarbonate. After drying and evaporation a residue was obtained. This was shown by T.L.C. to be an equal mixture of two compounds. The infrared spectrum showed the presence of a new carbonyl function at 1710 cm^{-1} and an obvious 5 membered lactone carbonyl at 1780 cm^{-1}. The original ester carbonyl at 1730 cm^{-1} was still present. The intensities of the different carbonyl groups were unequal and fell roughly into the order: lactone:ester:ketone = 1:1:2. Although the extinction coefficient of carbonyl groups in the infrared vary considerably, this fact coupled with the information from T.L.C. indicates the following structures (90) and (91) for the products.



(72) (90) (91)

This result is in keeping with the known lability of the epoxide function in acid media. Before abandoning this approach it was decided to examine the effects of shortening the reaction time. It is known that certain oxidation reactions such as Jones' reaction have very fast reaction times. It was therefore possible that ketone (91) was produced initially and then slowly converted into (90) as the epoxide was cleaved. Two facts support this hypothesis. Axial alcohols are oxidized faster than equatorial ones using Jones' reagent[79] and the opening of the epoxide is a slower process than oxidation.

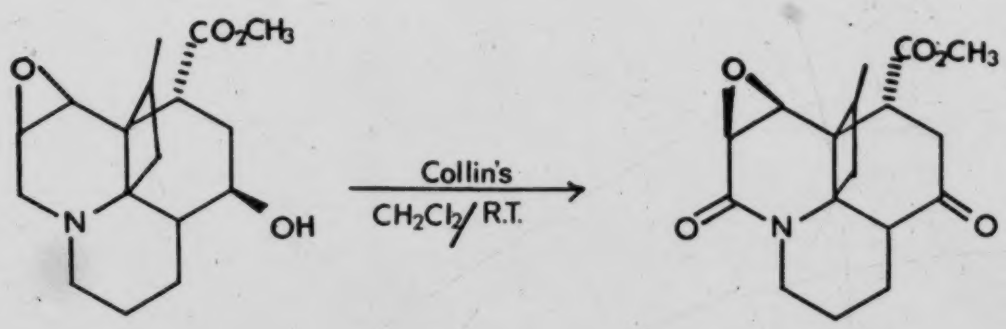
Accordingly the above reaction was repeated except that after 30 seconds the isopropanol was added and the reaction worked up as before. Examination by T.L.C. showed three components; namely the starting material (72)

and the two previous products (90) and (91). This prognosis was confirmed by infrared spectroscopy.

There is a modification of the Jones' reagent developed by Johnson[80] which is useful for the oxidation of sensitive substrates. This is a two phase method using an aqueous solution of chromic acid and a benzene solution of the substrate. Although the rate of migration of the substrate into the aqueous layer is slow, the reaction proceeds because of the fast rate of oxidation. Moreover the product is protected from the chromic acid by being reabsorbed into the benzene layer. This method was applied to methyl epiannotinate (72). Thus 1.5 equivalents of Jones' reagent was added to a solution of (72) in benzene and stirred rapidly for ten minutes. After quenching with isopropanol the reaction was worked up as before. The residue indicated three compounds as before; starting material (72) and the two ketones (90) and (91).

At this point we turned to those oxidation methods which involved neutral or basic reagents. Of all of these the most common and the simplest was Collins oxidation.[81] However a report by Marion, *et al.*,[82] indicated that the product of such a reaction might be a lactam (92). Partially out of curiosity and partially in the hope that this might not be so, we undertook to try this reaction. Treatment of methyl epiannotinate with 4 equivalents of Collins' reagent in methylene chloride for 30 minutes at room

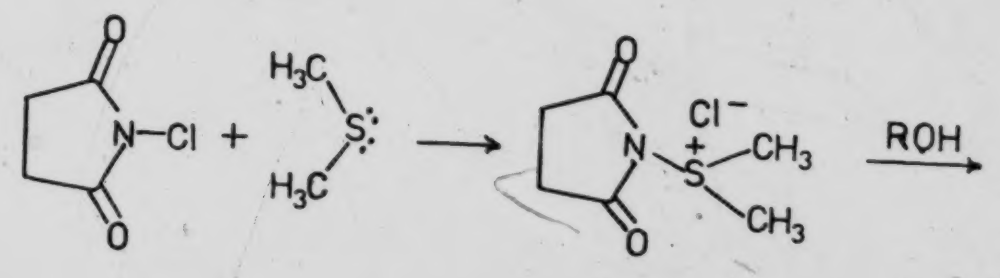
temperature gave after typical work up a single compound. This product showed a new carbonyl absorption at 1710 cm^{-1}. Unfortunately a prominent lactam carbonyl at 1650 cm^{-1} was also much in evidence. The product is thus (92).



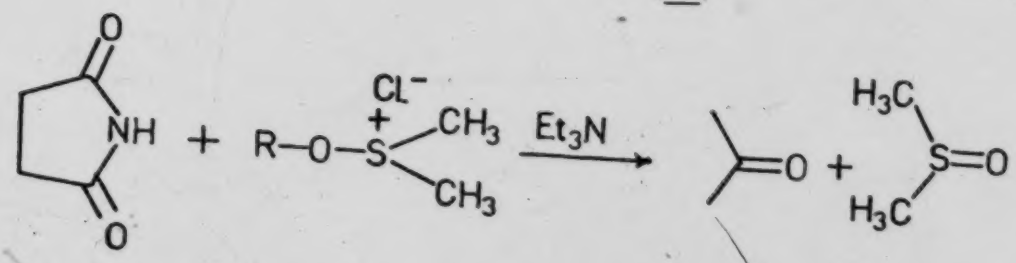
(72) (92)

Such a behaviour has been reported for Collins' reagent.[83]

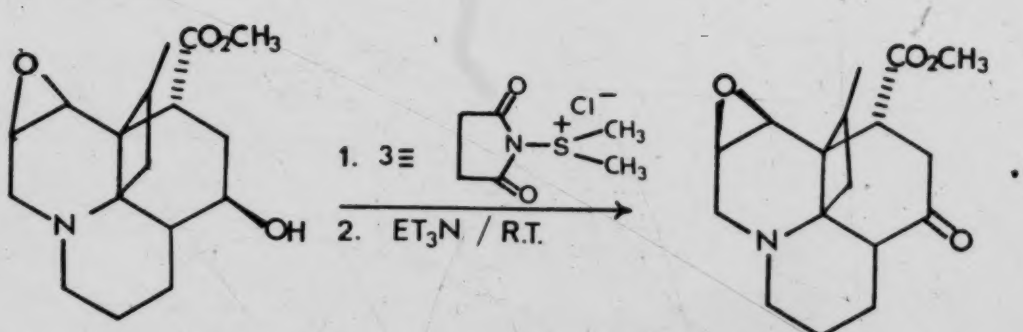
At this point we decided to make use of a neutral oxidizing agent. Such an agent was reported by Corey in 1972[84] and promised to be of value here. He treated N-chloro succinimide with one equivalent of dimethyl sulphide to obtain the complex (93). The latter on mixing with an alcohol, followed by triethylamine gives a carbonyl function.



(93)



Accordingly, a solution of the complex (93) was prepared in toluene and cooled to -25°C using a carbon tetrachloride/dry ice cryostat. Then 0.3 equivalent of methyl epiannotinate in methylene chloride was added and the mixture stirred at -25°C for two hours under a nitrogen atmosphere. After warming up to room temperature, triethylamine was added and the solution stirred for 20 minutes. Dilution of this solution with methylene chloride, followed by treatment with saturated sodium bicarbonate solution and evaporation gave a residue containing a single component. This was identified as the elusive ketone (94).

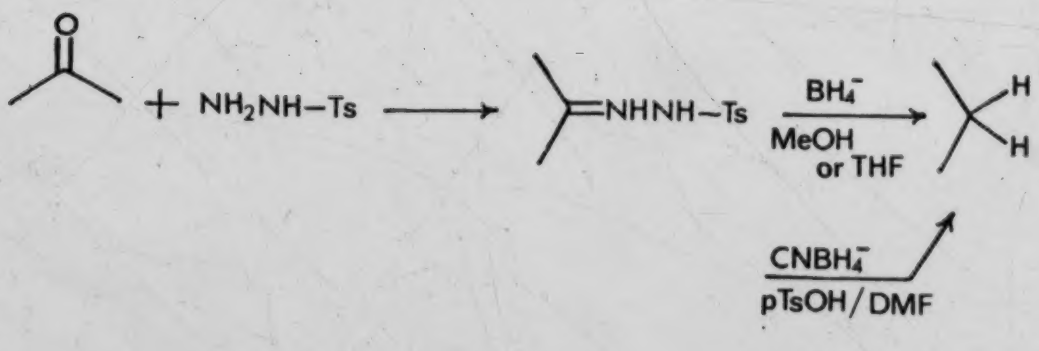


(72) (94)

Infrared spectroscopy showed a carbonyl absorption at 1730 cm^{-1} and 1709 cm^{-1}. The C.M.R. spectrum clearly indicated two carbonyl carbons and showed the loss of absorption due to C-5 at δ 58.2 in methyl epiannotinate. The molecular weight was confirmed by mass spectroscopy. The ketone could be crystallized from ether to give colourless rhombs M.P. 161-162°C.

Generally speaking there are two methods for reducing carbonyl groups to hydrocarbons. These are alkaline reduction such as Huang-Minlon and acidic reduction such as Clemmenson. Since the epoxide function of (84) is acid sensitive this effectively precludes all such acidic methods. Included here are those methods which involve acid catalysis such as dithiane preparation followed by Raney nickel. Thus we need only concern ourselves with non-acidic methods. Experience with lycopodine[85] had shown

that the C-5 carbonyl group is reduced only with great difficulty and elevated temperatures using standard hydrazine methods such as Wolf-Kishner or Huang-Minlon. What is more - these methods require the use of strong bases. Lately, however there have appeared a number of methods which involve an activated hydrazine such as tosylhydrazine and a metal hydride reducing agent. These are due to Cagliotti[86] and Hutchins.[87] Both methods involve preparation of a tosylhydrazine which is then reduced either by borohydride (á la Cagliatti) or cyano-borohydride (á la Hutchins).



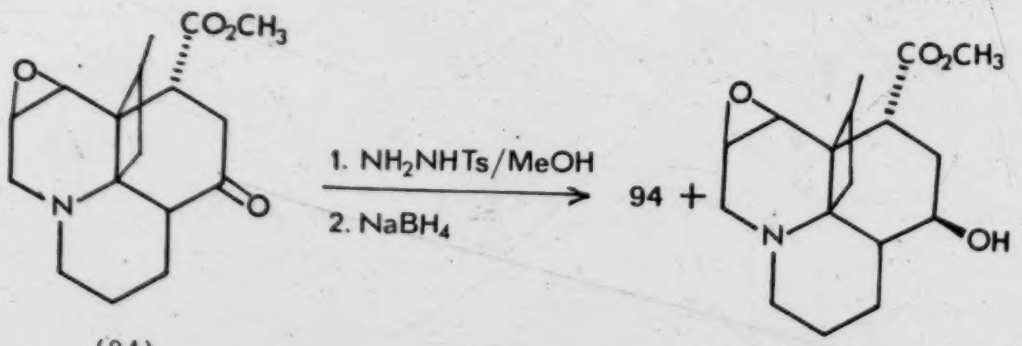
The use of cyanoborohydride requires the presence of an acid catalyst to form the imminium ion. In the absence of acid, cyanoborohydride lacks the nucleophilicity to

reduce the imine. We are thus confined to the Cagliotti method.

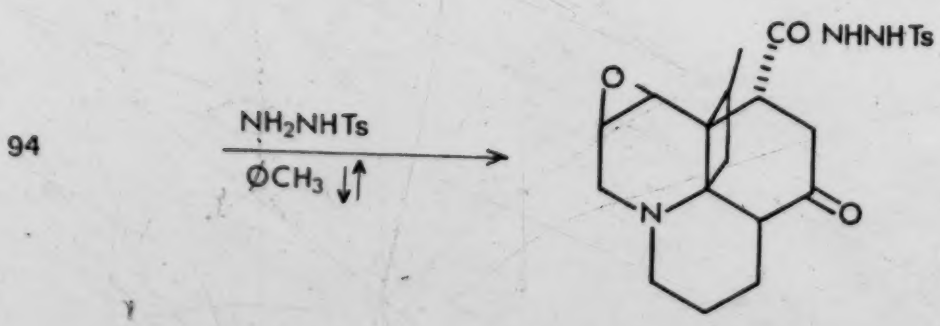
The ketone (94) was treated with 1.3 equivalents of tosylhydrazine in relfuxing methanol for one hour. After cooling to room temperature 10 equivalents of sodium borohydride was added and the mixture stirred overnight. Hydrolysis with saturated ammonium chloride, followed by extraction with methylene chloride and evaporation gave a residue consisting of three components. The major one of these was the starting material (94) and the second seemed to be identical with methyl epiannotinate (72). The minor component was not identified.

The meaning of this is clear, tosylhydrazone formation did not occur and the borohydride simply reduced the ketone (84) to the alcohol (62). It has been acknowledged that the tosylhydrazone formation is difficult for hindered ketones[88] and refluxing overnight in absolute ethanol has been recommended to expedite the matter. On trying this method a new less polar compound could be detected after 24 hours, by analysis with T.L.C. on silica gel. The picture was far from clear. The new compound was a large diffuse spot and its appearance varied considerably on subsequent T.L.C. analysis. It also gave a positive test with 2.4 D.N.P. spray. Evaporation of the ethanol, followed by extraction of the residue with methylene chloride and water gave only starting

material (94) in good yield. No trace of the new compound could be detected. This puzzling result was avoided by switching to a non-polar solvent such as toluene.



(94) (62)

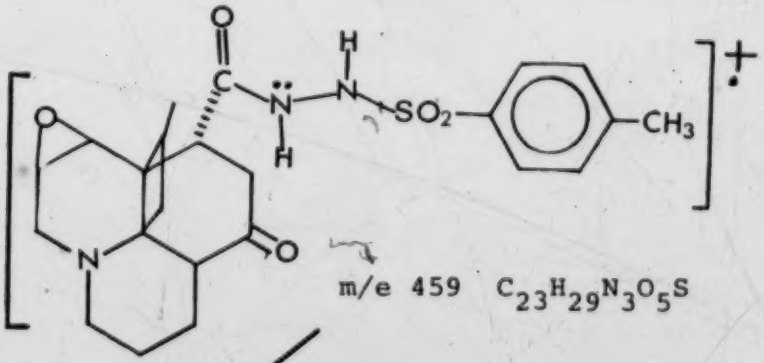


(95)

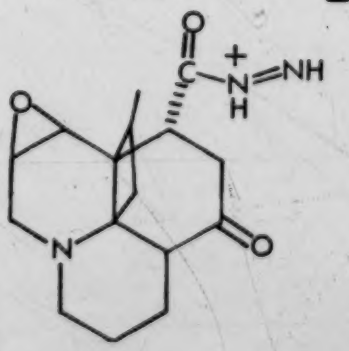
Refluxing a solution of (94) in toluene results in no change after 24 hours. The ketone can be recovered intact. If 3 equivalents of tosylhydrazine is added and refluxing is continued for a further 24 hours a new,

considerably more polar, compound is formed. This survives aqueous work up and can be purified by chromatography on silica gel.

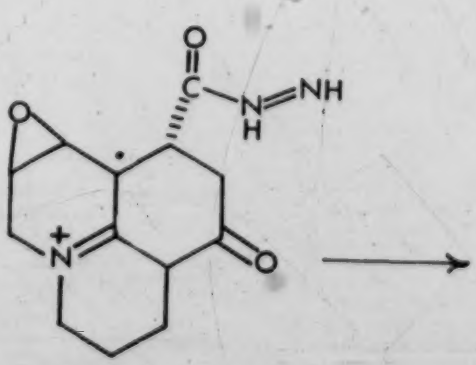
P.M.R. spectroscopy of this compound indicates the incorporation of an aromatic nucleus. The aromatic proton and methyl group are clearly visible. What is interesting however is that the carbomethoxy group has gone. This is borne out by the infrared spectrum. This still contains the ketone at 1710 cm^{-1} but the carbonyl due to the ester at 1730 cm^{-1} in (94) is absent. Furthermore two weak absorptions due to the amide group appear at 1600 cm^{-1} and 1690 cm^{-1}. All of this is further confirmed by the mass spectrum.



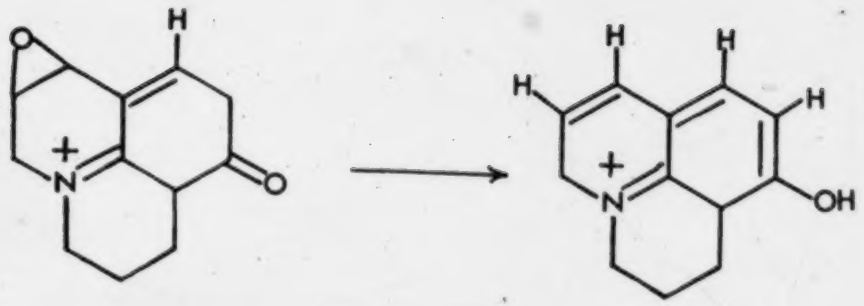
m/e 459 $C_{23}H_{29}N_3O_5S$



m/e 304 $C_{16}H_{22}N_3O_3$



m/e 262 $C_{13}H_{16}N_3O_3$

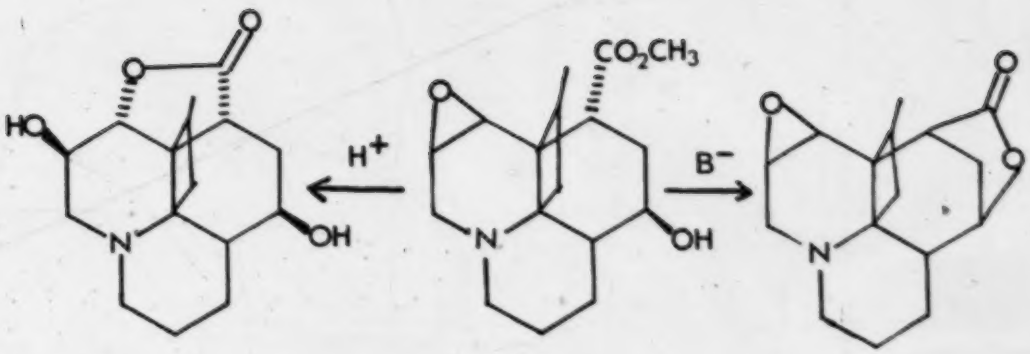


m/e 204 $C_{12}H_{14}NO_2$ m/e 188 $C_{12}H_{14}NO$

metastable @ 172.5 $\Longrightarrow$ 204 → 188

With this result no further attempt was made to investigate the reduction of the ketone (94).

There remained one further possibility to achieve our synthetic aim. This involved blocking the hydroxyl function with a suitably inert group. Thus if the hydroxy group could not be removed, it would be rendered inactive. Such an approach did not seem favourable however as a previous experience had shown any acid catalysed reaction was impractical and most base catalysed reactions would lead to annotinine, e.g.,



It was decided to try a number of examples of both sorts of reaction to see if these difficulties could be overcome.

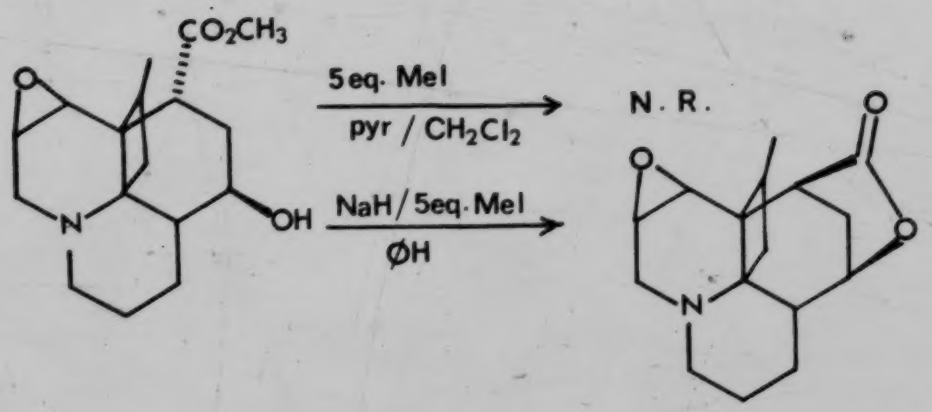
A solution of methyl epiannotinate and excess dihydropyran using p-toluene sulphonic acid as catalyst was stirred under reflux for 30 minutes. At this point no starting material could be detected, there being one new product less polar than starting material and corresponding to annotinine hydrate. Evaporation of the solvent gave a residue which contained a single alkaloid with an infrared absorption at 1775 cm^{-1}. Since this also corresponded with annotinine hydrate no further action was taken.

Any base catalysed blocking group, if it was to be stable to the strong base conditions required for the formation of ester "M", would have to be formed under

non-reversible conditions. This essentially removed all ester type blocking groups from consideration, except perhaps mesitoic esters. It is doubtful if the latter could be inserted at the hindered C-5 of methyl epiannotinate. The requirement of non-reversible formation leads to the use of ethers, of these, there are quite a few which are regularly used as blocking groups. These are methyl, methoxy methyl, trimethylsilyl and dimethyl t-butyl silyl. All of these seemed suitable for the task in hand except perhaps trimethylsilyl which is cleaved in strong base. It was decided to investigate this route.

Reaction of methyl epiannotinate with 5 equivalents of methyl iodide in methylene chloride using pyridine as catalyst gave after 24 hours at room temperature only starting material. No quaterization at nitrogen took place, which gives some indication of the steric hindrance at this site. Refluxing the solution for a similar amount of time did not change the outcome.

Switching to a stronger base, such as sodium hydride in benzene, with 5 equivalents of methyl iodide present, gave only annotinine as product.

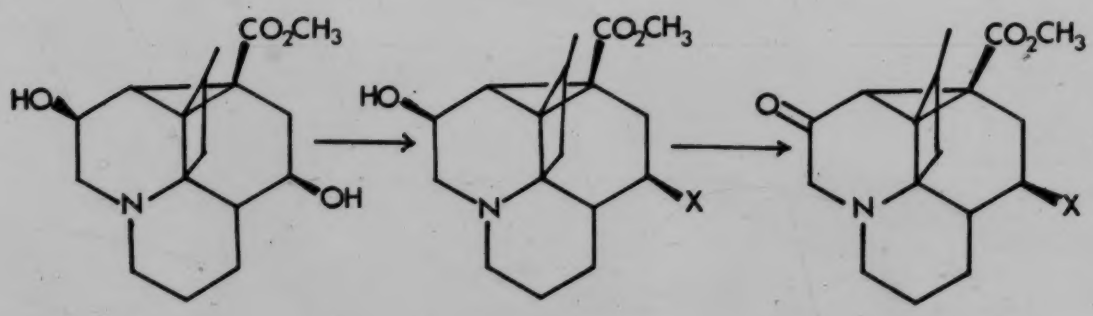


Chloromethyl methyl ether is an even better electrophile than methyl iodide. It was felt that the use of this reagent might trap any alkoxide formed before it can close internally to give annotinine. Repetition of the above experiments using chloromethyl methyl ether instead of methyl iodide gave identical results. Again no quaternization at nitrogen could be detected.

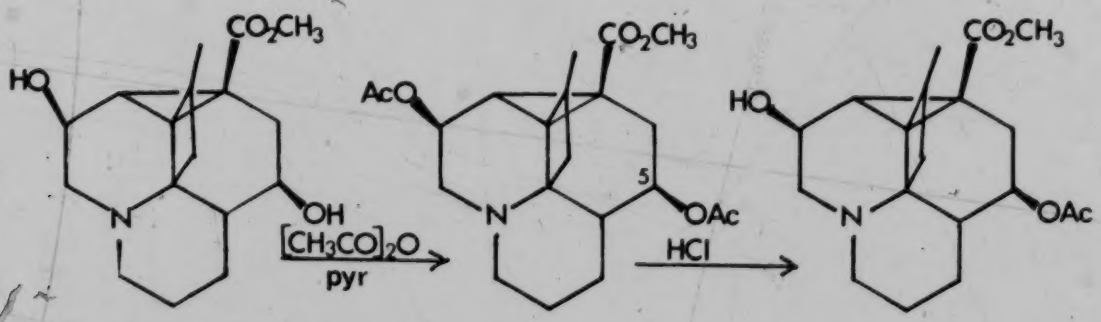
There remained only at this point the dimethyl t-butylsilyl blocking group. Corey reports this to be stable to basic reaction conditions and yet easily removed by treatment with tetra-n-butyl ammonium fluoride in tetra hydrofuran.[89] He also described a procedure for its reaction with alcohols which involved imidazole in dimethyl formamide. This latter aspect seemed very useful in light of our earlier experiences with strong bases such as hydride.

Thus treatment of methyl epiannotinate with dimethyl t-butylsilyl chloride (1.5 equivalents) and imidazole (3 equivalents) in dimethylformamide at 35°C gave after 24 hours only starting material. Heating the reaction mixture to 80°C for 12 hours showed only the production of annotinine. Beside starting material no other products could be detected.

The aim of the preceeding experiments was to prepare a derivative of methyl epiannotinate in which the C-5 hydroxy function had either been removed or blocked by a suitably inert blocking group. The failure to achieve any success in this endeavour prompted us to consider other routes to a suitable oxidized ester "M" derivative. Thus we chose to examine path (1) as referred to on page (132).



Some work had already been done on this route by Valverde-Lopez[90] who prepared ester "M" diacetate and from this the axial monoacetate by hydrolysis of the diacetate with dilute hydrochloric acid.



This procedure takes advantage of the highly hindered nature of C-5 position of the annotinine nucleus. However the yield of both these reactions is poor and keeping in mind the limited quantities of ester "M" we had by this time, we considered the improvement of these procedures.

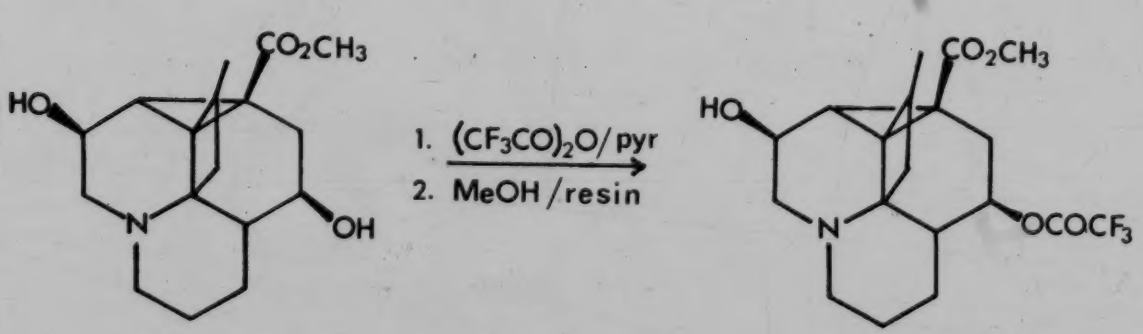
In the formation of the diacetate and its subsequent hydrolysis to the monoacetate the reaction rate is slow and the long reaction times required cause serious decomposition and formation of biproducts. Thus purification by column chromatography is required after each step.

If the acetic anhydride were replaced by a higher energy compound the reaction rate would be speeded up. Furthermore if this reagent had a bulky nature it might

increase the free energy differences between the two possible mono derivatives.

These requirements led us to consider trifluoroacetic anhydride and trichloroacetyl chloride.

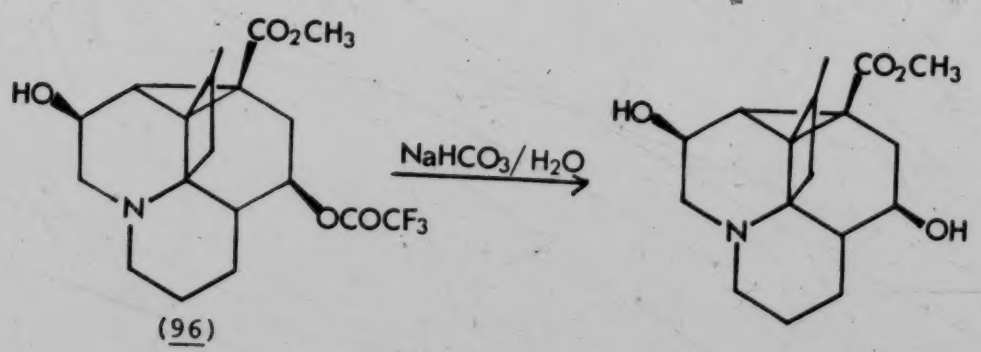
The reaction of ester "M" and excess trifluoroacetic anhydride and pyridine is nearly instantaneous. At room temperature the ester "M" dissolves within 2 or 3 minutes. The reaction mixture is left overnight and then diluted with methanol. This solution is then passed through a weak acid ion-exchange resin (H^+ form) that has been thoroughly flushed with anhydrous methanol. After evaporation the residue is treated under high vacuum to remove traces of pyridine. The product is ester "M" 5-monotrifluoroacetate (96).



(96)

It can be assumed that the ditrifluoroacetate is produced and is converted to the mono derivative during

work up. (96) is a sensitive compound and is gradually hydrolysed by moisture. On treatment of (96) with diluted $NaHCO_3$ ester "M" is produced almost immediately and precipitates out.



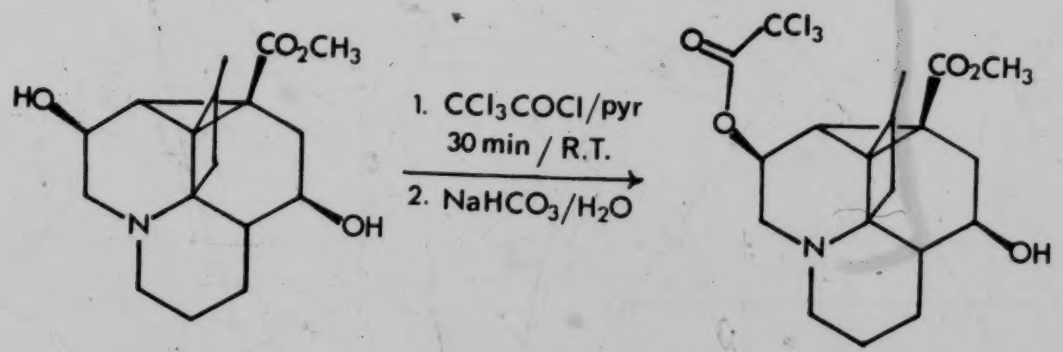
(96)

The beauty of this procedure is that ester "M" can be converted to the required mono derivative in 5 minutes and in good yield and excellent purity. No chromatography is required. The monotrifluoroacetate was considered to be to sensitive to moisture, however, and we decided to investigate the monotrichloro acetate derivative instead.

The reaction of x.s. trichloroacetylchloride with ester "M" and pyridine is quite different from the preceeding one with trifluoroacetic anhydride.

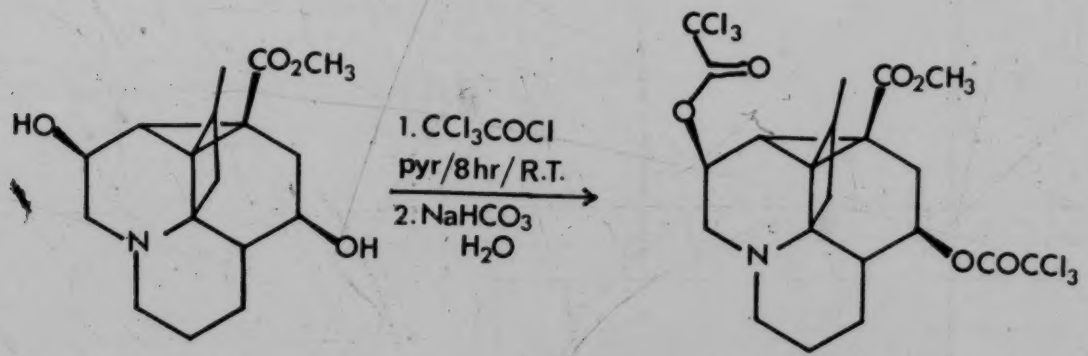
At room temperature the ester "M" dissolves within 30 minutes. If the reaction is stopped by pouring in a large x.s. of aqueous $NaHCO_3$ solution the product obtained by extraction with methylene chloride and evaporation is

the equatorial monotrichloroacetate (77).



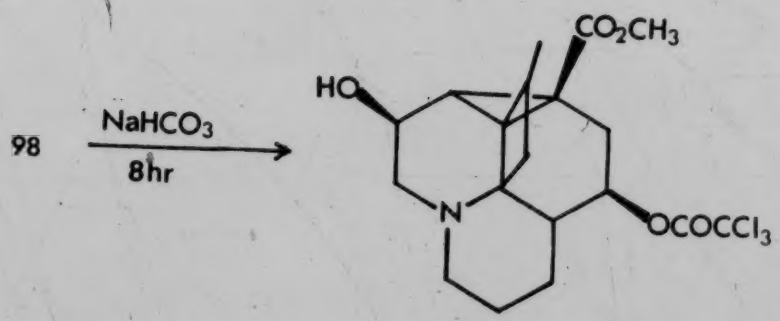
(63) (97)

If on the other hand the reaction is allowed to proceed overnight, followed by similar work up, the product is the ditrichloroacetate (98).



(63) (98)

The ditrichloroacetate can be converted to the axial monotrichloroacetate (99) by vigourously stirring a methylene chloride solution of the former with aqueous $NaHCO_3$ overnight.



(99)

The structures of all of these compounds were corroborated by a combination of various spectral properties.

The monotrifluoroacetate (96) was identified by its mass spectrum. It gave a clear parent peak at m/e 403 ($C_{19}H_{24}O_5NF_3$). It also showed a peak at m/e 344 corresponding to loss of the carbomethoxy group. This type of cleavage rather than the loss of the bridgehead cyclobutane ring is indicative of ester "M" derivatives. The infrared spectrum indicated a hydroxy group at 3590 cm^{-1}, a trifluoroacetoxy group at 1785 cm^{-1} and a cyclopropyl ester group at 1720 cm^{-1}. The P.M.R. spectrum indicated a single broad multiplet at δ 5.0 which has been indentified as the C-5 methine proton in various ester "M" derivatives.

The equatorial trichloroacetate (97) was identified by its P.M.R. spectrum which showed a sharp sextet centered at δ 4.9 corresponding to the methine proton at C-10.

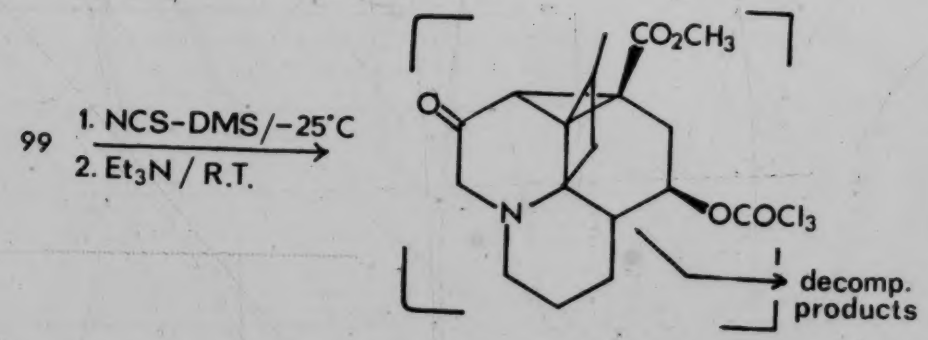
The infrared spectrum showed a small sharp hydroxy peak at 3600 cm^{-1}, a trichloroacetoxy peak at 1765 cm^{-1} and a carbomethoxy peak at 1715 cm^{-1}. Although no great diagnostic value can be put on e_{max} values in the infrared it is useful to note that the acetoxy and carbomethoxy groups were roughly of equal intensity. A detailed C^{13} M.R. spectrum also agreed with this structure, in that the C-10 doublet has been shifted downfield 11 ppm from that of ester "M".

The ditrichloroacetate (98) showed in its infrared spectrum no hydroxy peaks, but instead the trichloroacetoxy peak at 1765 cm^{-1} was double the intensity of the carbomethoxy peak at 1720 cm^{-1}. The P.M.R. spectrum showed a broad singlet at δ 5.15 and a sextet at δ 4.8. These correspond respectively to the C-5 and C-10 methine protons.

The axial monotrichloroacetate (99) gave a mass spectrum with a parent peak at m/e 451 ($C_{19}H_{24}O_5NCl_3$). It showed a characteristic peak at P+2 of equal intensity to the parent peak. This was indicative of the three chlorine atoms. As with other ester "M" derivaties the main fragmentation pattern involved loss of 59 m.u. due to the carbomethoxy group. The infrared spectrum showed a broad hydroxy group at 3300 cm^{-1} ($CHCl_3$ solution) and two carbonyls of equal intensity at 1768 cm^{-1} (CCl_3CO-) and 1725 cm^{-1} ($-CO_2Me$). The P.M.R. showed a single broad

peak at δ 5.15 due to the C-5 methine.

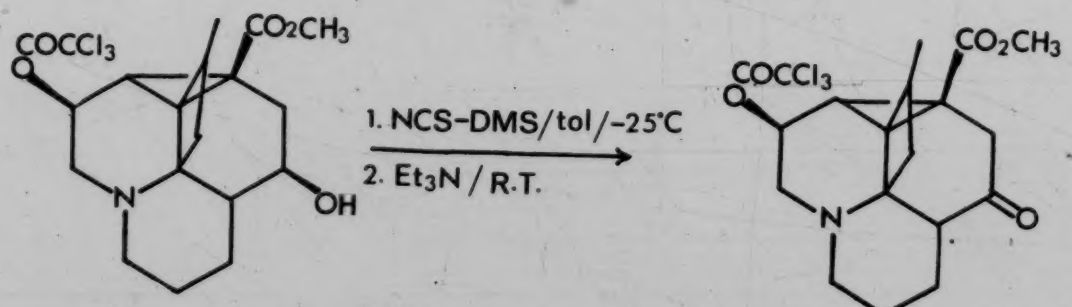
Oxidation of the axial trichloroacetate (99) was attempted using Corey's method involving the N-chlorosuccinimide/dimethyl sulphide complex. The starting material was consumed in the course of the reaction but no identifiable products could be obtained. Extensive decomposition was observed. Various combinations of lower temperatures and shorter reaction times were tried but the only effect was to produce more remaining starting material. If the desired ketone is being produced it is being oxidized further even faster than it is being produced.



(99)

Similar results were obtained using Jones' reagent and modified two phase Jones' reagent.

On the off chance that the trichloroacetate function could be involved in this setback it was decided to repeat the reaction using the equatorial monotrichloroacetate as starting material. Oxidation of the latter with Corey's reagent proceeded smoothly and gave the ketone (100) in good yield and purity.

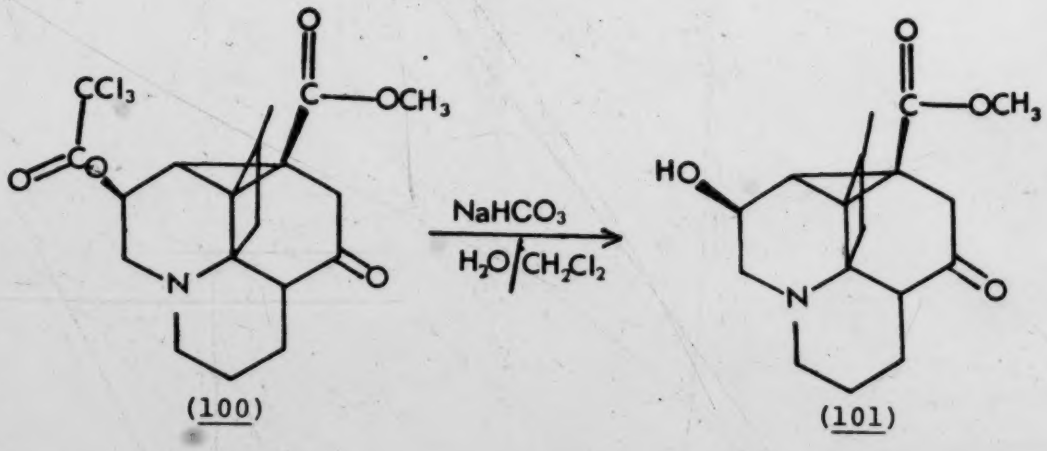


(97) (100)

This clearly rules out any interference by the trichloroacetyl function in the oxidation of the axial monotrichloroacetate.

The structure of the ketone (100) was clearly indicated by spectral evidence. The C.^{13}M.R. spectrum shows a carbonyl carbon at 210.5 ppm. The band at 70 ppm which is characteristic of this carbon in the parent monotrichloroacetyl compound (97) is absent. The infrared shows an additional carbonyl when compared to the monotrichloro compound (97) at 1710 cm^{-1}. This carbonyl appears as a shoulder on the carbonyl at 1725 cm^{-1} (CO_2Me). The mass spectrum has a parent peak at m/e 449 ($C_{19}H_{22}O_5NCl_3$) with a peak of equal intensity at m/e 451 (P + 2) due to the three chlorine atoms. Interestingly enough the main fragmentation route for this molecule is loss of methyl to give a large m/e 434 peak.

This ester (100) could be hydrolysed to the hydroxy ketone (101) by vigourously stirring a mixture of (100) in CH_2Cl_2 and a solution of saturated aqueous $NaHCO_3$ overnight. The hydroxy ketone is recovered as the sole product.

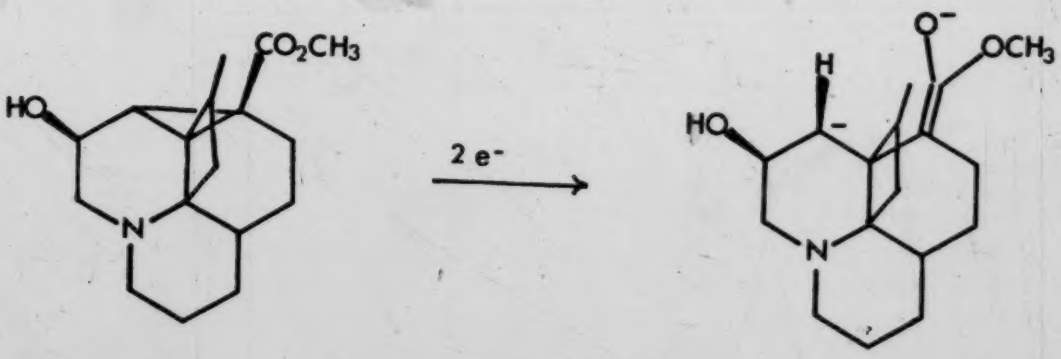


(100) (101)

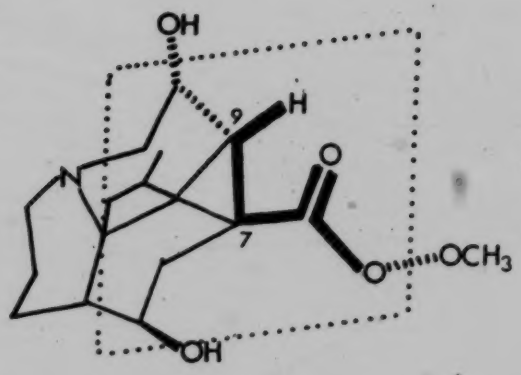
Spectral evidence for this new compound (101) is as follows. The infrared shows only one carbonyl region at 1710 cm^{-1} (broad). The previous band at 1765 cm^{-1} due to the trichloroacetyl function has disappeared. The C.^{13}M.R. spectrum shows at C-10 the characteristic up-field shift of 8.8 ppm, compared to (100), observed when an ester is hydrolysed to an alcohol. A similar shift is observed in the P.M.R. spectrum for the triplet due to the methine proton at C-10. Addition of trichloroacetyl-isocyanate to the N.M.R. tube reversed this observation. The mass spectrum confirms the molecular formula $C_{17}H_{23}NO_4$. The base peak corresponds to the loss of a methyl group (confirmed by exact mass measurements). This fragmentation was also observed for the ester (100). As no such fragmentation was observed in the ketone derived from methyl epiannotinate (94), it seems that loss of a methyl

group to form the base peak is indicative of the ester "M" structure in such ketones as (100) and (101).

The failure of the axial monotrichloroacetate (99) to give the desired ketone (99A) led us to consider other possibilities. The ketone had been desired so that the nature of the cyclopropane ring could be established by reducing it with lithium in ammonia. It occurred to us at this point that perhaps the cyclopropane ring could be reduced without the presence of the ketone. As the latter serves as an electron sink it is also possible that the ester function at C-7 could serve the same purpose, e.g.,



Furthermore the stereoelectronic control observed in similar reductions of cyclopropyl ketones (70) should still apply, as the rotation of this ester is severely restricted by the cyclobutane ring. Of the possible conformations the most favourable is one in which the carbonyl function and the C7-9 bond lie in the same plane.

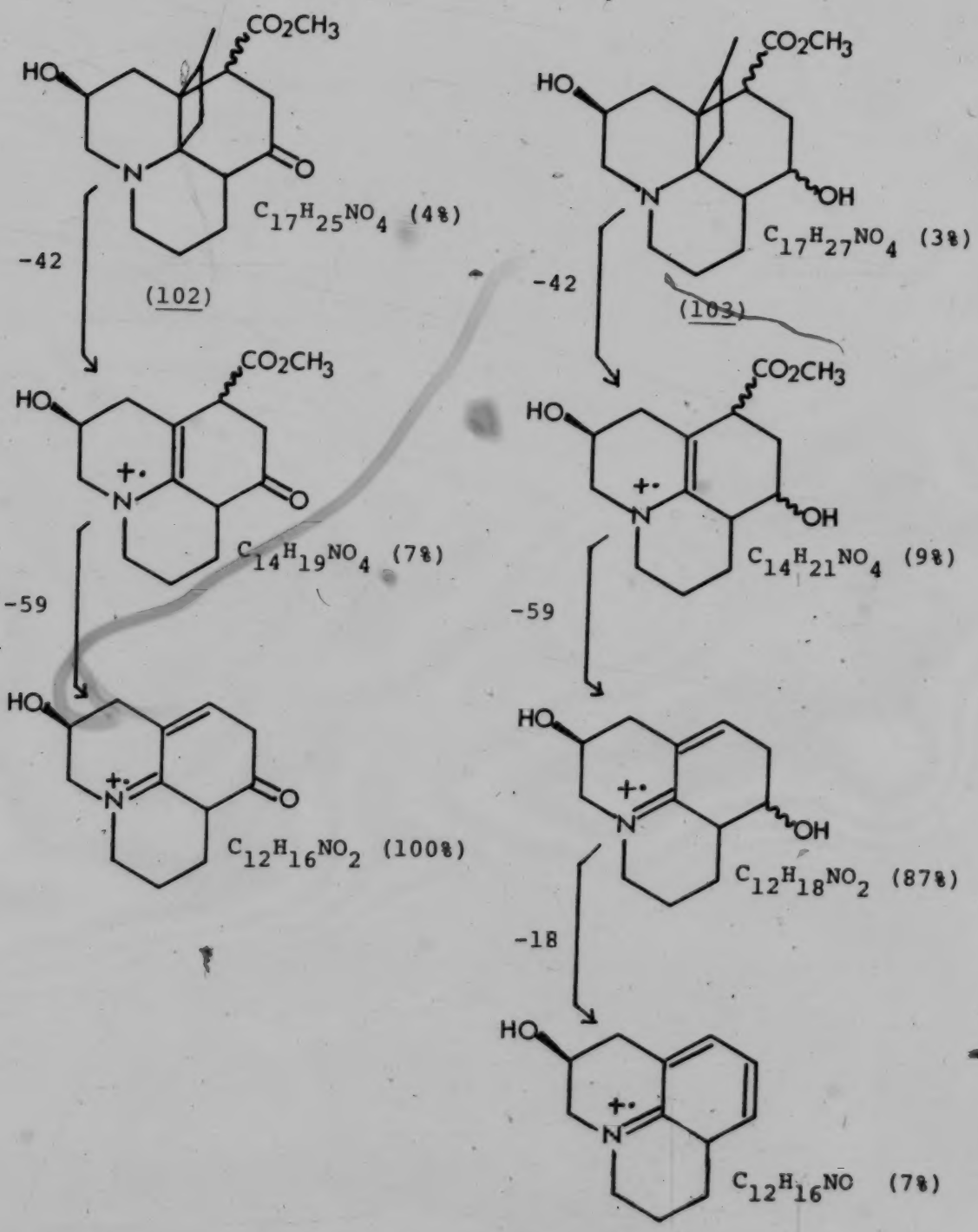


This conformation exactly satisfies the orthogonality requirements established by earlier workers.[91]

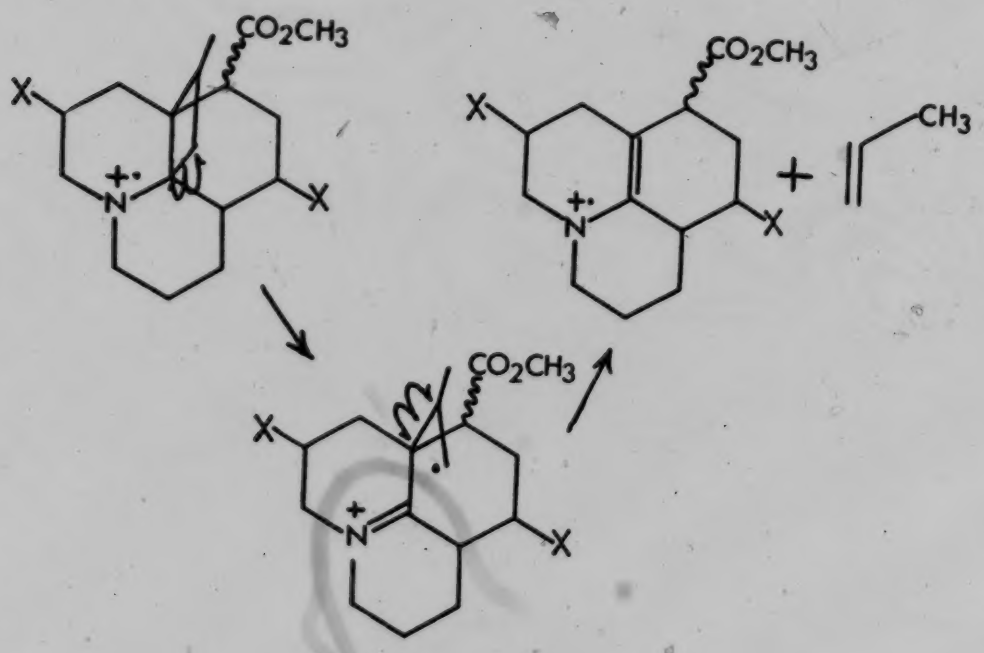
Therefore it seemed reasonable that reaction of ester "M" or a derivative thereof with lithium in ammonia should reduce the cyclopropane ring preferably across the C7-9 bond.

Since ester "M" itself is extremely insoluble in most solvents there seemed little point in trying out the experiment on it. Instead the hydroxy ketone (101) was used instead. It was on hand from the previous series of experiments and it was quite soluble in T.H.F. Accordingly a solution of lithium in freshly distilled

(over sodium) ammonia was prepared and a T.H.F. solution of the hydroxy ketone (101) added. This ammonia solution was let reflux for 30 minutes and then the reaction was stopped by adding a large excess of ammonium chloride. The ammonia was evaporated and the residue taken up in a mixture of ether and water. The ether layer, (after washing and drying) gave, upon concentration, a residue consisting of two compounds. These were identified by their mass spectra as the hydroxy ketone (102) and the diol (103). In particular both compounds showed fragmentation patterns analogous to methyl epiannotinate (72).


CO2CH3
HO
N
O
C17H25NO4 (4%)
-42
(102)
+·
C14H19NO4 (7%)
-59
C12H16NO2 (100%)
OH
C17H27NO4 (3%)
(103)
C14H21NO4 (9%)
C12H18NO2 (87%)
-18
C12H16NO (7%)

The fact that the characteristic loss of 59 observed in all ester "M" derivatives is notably absent in both of these compounds is proof that the cyclopropane ring has been cleaved. Moreover the distinctive loss of a methyl group observed in the ester "M" ketones (100) and (101) is definitely absent in (102). It would seem that the loss of 42 from the parent peak is characteristic of the abscence of the cyclopropane. The lone pair on the nitrogen should ionize preferentially and this ion should result in the loss of cyclobutane ring. However in ester "M" derivatives the presence of the cyclopropane ring prevents the formation of the $\Delta^{12,13}$ double bond.



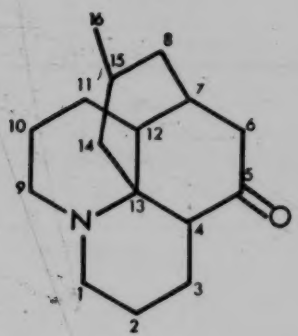
The coplanarity of carbons 7, 12 and 8 required for the $\Delta^{12,13}$ double bond is prevented by the cyclopropane ring.

Attempts to separate the mixture of (102) and (103) were unsuccessful due to the small quantities available.

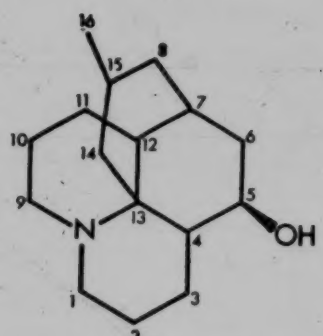
The reduction was repeated using a large excess of lithium and with the addition of t-butanol as a proton donor.[92] After work up, the product was found to be a single compound which was identified as the diol (103). The infrared spectrum of (103) shows the presence of two hydroxy groups at 3400 cm^{-1} and 3510 cm^{-1}. The ester carbonyl is found at 1735 cm^{-1}. This value is clear evidence that the cyclopropane ring has been reduced. In *all* ester "M" derivatives examined the value for this ester carbonyl was about 1718 cm^{-1}. The P.M.R. spectrum of (103) shows a two proton multiplet at δ 3.9. Addition of trichloroacetyl isocyanate to the N.M.R. tube results in the shifting of this multiplet to δ 5.2. Thus it can be stated that compound (103) has the structure indicated and this is further proof that the structure shown for ester "M" (72) is also correct.

Futher evidence for the structure of ester "M" comes from the study of the carbon-13 magnetic resonance spectra of the derivatives made in the course of this investigation. The assignment of the various resonances was facilitated by work already accomplished in this group on the lycopodine series of alkaloids.[93] Thus

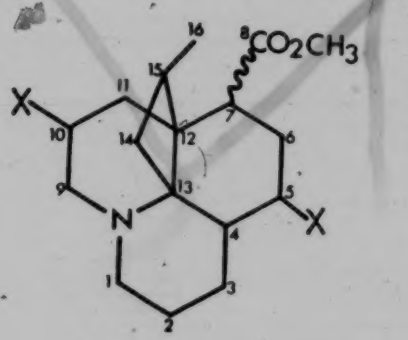
the assignment of the resonances due to carbons 1, 2, 3, 4, 5, 9 and 13 was based on those observed in lycopodine (70) or dihydrolycopodine (104).



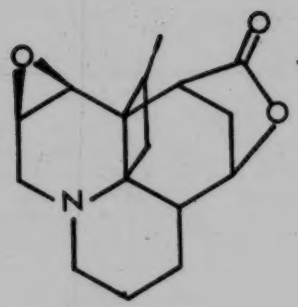
(70)



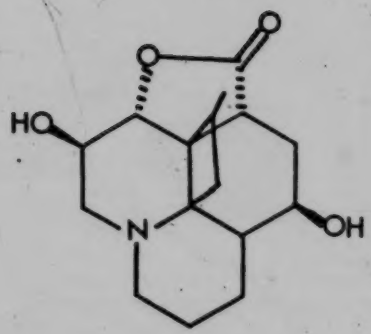
(104)



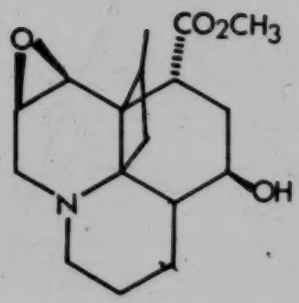
The assignment of the resonances due to carbons5, 6, 10 and 11 was easily achieved using the shifts effected at these sites by various chemical transformations at C-5 and 10. Carbon-16 was easily assigned as the only high field methyl group. The resonance due to carbon-7 was determined by its diminution after exchanging the hydrogen at that position with deuterium. This left only carbons 14 and 15 and these were assigned as the only remaining resonances. By this means the following values were determined for the various carbons in several annotinine derivatives.



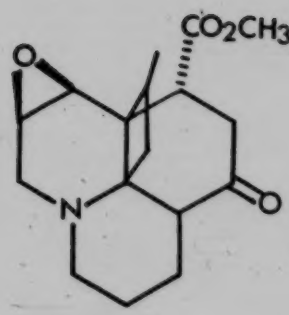
(71)



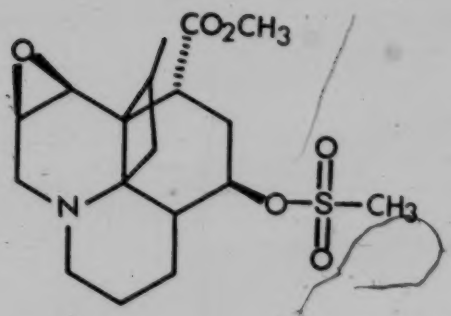
(75)



(72)



(94)



(89)

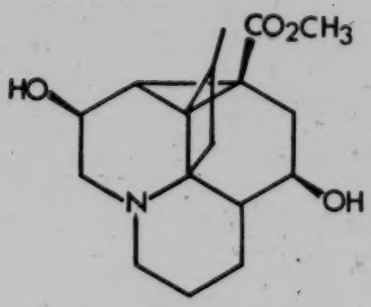
carbons	(71)	(75)	(72)	(94)	(89)[c]
1	43.8	47.4	43.8	44.5	43.8
2	22.6	23.6	21.3	21.1[b]	20.9
3	25.2	29.3	25.1	19.8[b]	25.0
4	39.9	34.1	34.2	44.6	33.6
5	80.4	65.8	69.1	209.4	80.5
6	35.3	33.2	34.6	39.6	30.8
7	43.8	40.1	37.8	41.8	38.6
8	178.6	178.2	175.1	174.8	173.9
9	46.7	49.6	47.5	48.0	47.5
10	52.8[a]	65.2	51.6	54.1[a]	51.9[a]
11	51.4[a]	78.6	51.6	52.1[a]	51.4[a]
12	44.5	45.4	41.7	39.9	41.6
13	58.3	58.3	57.8	58.6	57.6
14	35.2	29.3	32.7	35.8	33.6
15	31.8	30.1	33.2	30.8	30.8
16	12.4	14.6	16.2	14.0	16.2
17			51.6	52.2	51.4
SO_2Me					38.6

a. C-10 and C-11 may be reversed.

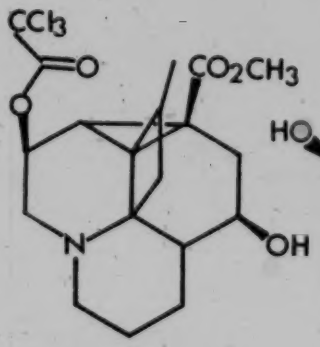
b. C-2 and C-3 may be reversed.

c. insufficient quantities available for detailed off resonance studies.

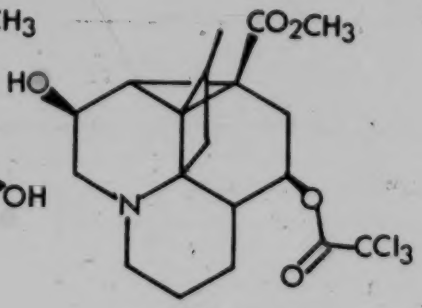
The carbon-13 magnetic resonance spectra of five ester "M" derivatives were then measured. The assignments of the various resonances were carried out in a similar fashion as for the annotinine derivatives. Thus carbons 1, 2, 3, 4, 5, 9 and 13 were assigned on the basis of the lycopodine and annotinine values. Carbon-7 was assigned to a new high-field singlet not observed in either annotinine or lycopodine. This singlet was confirmed by modulated off-resonance experiments. Carbons 5, 6, 10 and 11 were assigned on the basis of the shifts observed on changing the functionalization at carbons-5 and 10. Thus the following values were observed for the resonances in question.



(73)



(97)



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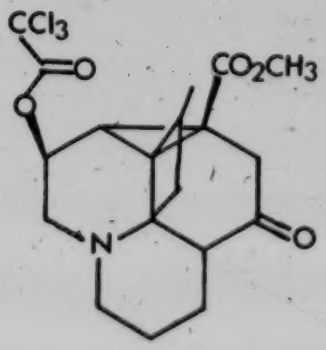
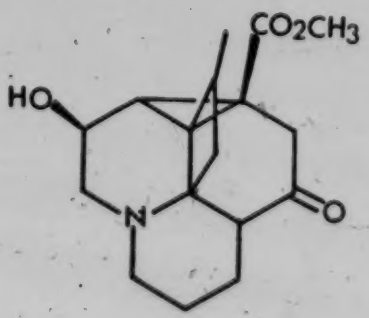


(100)



(101)

carbons	(73)	(99)[b]	(97)	(100)[b]	(101)
1	47.8	48.4	46.9	46.1	48.5
2	21.0[a]	21.2	22.1[a]	20.5	19.6
3	21.5[a]	20.4	22.9[a]	18.3	18.8
4	38.6	37.2	40.2	49.6	49.7
5	68.5	76.2	68.8	210.5	209.2
6	29.2	25.2	30.1	35.3	34.6
7	28.3	27.7	27.9	28.9	29.2
8	173.4	170.1	173.3	170.8	172.6
9	49.5	50.8	47.5	52.5	53.6
10	59.6	58.8	70.2	69.9	64.0
11	32.4	31.9	28.9	33.9	37.0
12	37.5	37.2	38.8	39.7	38.3
13	61.3	60.4	55.7	63.6	58.5
14	34.0	33.7	36.0	31.7	32.6
15	28.5	28.5	28.4	28.9	29.9
16	16.9	16.2	16.8	16.7	18.6
17	52.5	50.8	52.2	52.5	52.2
$COCCl_3$		161.6	161.6	161.6	

a. C-2 and C-3 may be reversed.

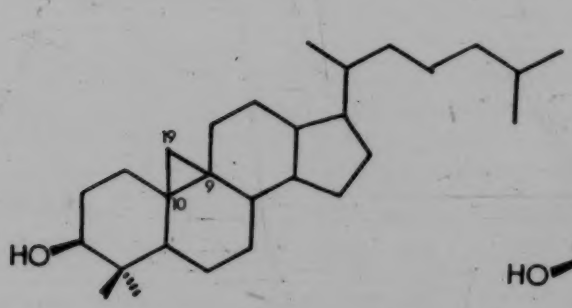
b. insufficient quantities available for detailed off-resonance studies.

Thus by comparing the values for C-7 in the annotinine series and the ester "M" series it can be seen that C-7 experiences an upfield shift from an average value of 40.4 in the annotinine series to an average value of 28.4 in the ester "M" series. If one ignores the value for annotinine hydrate (75) the average value for C-11 in the annotinine series is 51.6. In the ester "M" series the average value for C-11 is 32.8. The change at C-7 is 12 ppm and at C-11 it is 18.8 ppm. The value for the upfield shift at C-7 is smaller because this carbon is changing from a tertiary to a quaternary center.

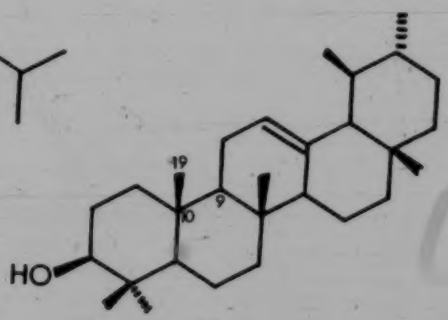
The average value for C-12 in the annotinine series is 42.6 whereas in the ester "M" series the average value for C-12 becomes 32.3. This represents an upfield shift of about 12 ppm. Since carbon-12 is fairly remote from any changes in functionalization this upfield shift must be mainly due to the presence of the cyclopropane ring. There can be no doubt about the values of the resonance observed for C-12 as it is a singlet and easily distinguished from C-13 or C-7.

It is difficult to account for these strong upfield shifts without invoking the presence of a cyclopropane ring. In particular the upfield shift observed for C-7 is directly contrary to the shift expected on going from a tertiary center to a quaternary center. Thus for example, C-10 in cycloartenol compounds[94] (105) occurs

at 26.0 whereas in most tetracyclic or pentacyclic triterpenes, e.g., β-amyrin[95] (106) it occurs at about 37.0.



(105)



(106)

Here the upfield shift on becoming part of a cyclopropane ring is 11 ppm. Similarly the observed upfield shift for C-9 on going from (106) to (105) is 27 ppm.

In conclusion it can be said that the carbon-13 magnetic resonance spectra of ester "M" derivatives are in keeping with the presence of a cyclopropane ring about carbons-7, 11 and 12.

EXPERIMENTAL

Melting points were determined on a Fischer-Johns hot stage melting point apparatus and are uncorrected.

Infrared spectra were recorded on a Perkin-Elmer Model 421 dual grating infrared spectrophotometer.

Proton magnetic spectra were measured using a Varian Associates Model HR-100 spectrometer with tetramethylsilane as standard.

C.M.R. spectra were measured at 22.63 MHz in the Fourier mode using a Bruker HFX-90 spectrometer in conjunction with a Nicolet-1085, 20K computer.

Mass spectra were recorded on an A.E.I. Model MS-50 or MS-9 mass spectrometer. O.R.D. and C.D. were determined on a JASCO O.R.D./C.D., SS-20-2 (modified).

U.V. spectra were recorded on a Cary Model 15 U.V. and visible spectrometer.

Thin layer chromatography was performed using Merck Silica Gel G (Type 60) with 1% General Electric P-1 Electronic Phosphor added.

Isolation of annotinine from *Lycopodium annotinum*.

Lycopodium annotinum plants were collected near Medicine Lake in Jasper National Park. The plants were air dried for 10 days and then milled to yield a fine powder (15 kgms approximately). This powder was extracted with methanol using a large Soxholet apparatus. The

methanol extract was concentrated *in vacuo* to yield a dark green sludge. This sludge was stirred overnight with 5% HCl and the resulting liquid decanted. This procedure was repeated twice. The combined acidic extracts were then continously extracted with chloroform for 2 days to remove fats and other neutral materials.

The resulting aqueous acidic layer was then basified with concentrated ammonium hydroxide while cooling was maintained with ice. This aqueous solution was again continuously extracted with chloroform for 2 days. Evaporation of the chloroform *in vacuo* yielded a dark brown powder. The powder was dissolved in the minimum amount of boiling ethanol. The latter upon cooling deposited crystals of annotinine (700 mgms). A further quantity (150 mgms) could be obtained by chromatography on alumina of the residue resulting from concentration of the mother liquor.

M.P. 228-9°C (lit. 232°C).

Treatment of annotinine (71) with sodium methoxide

Freshly cut sodium (20 mgms, 0.9 mmoles) was dissolved in 50 mls of dry methanol (dried by distillation from magnesium). After cooling to room temperature annotinine (30 mgms, 0.1 mmoles) was added and the mixture stirred continuously at room temperature. Aliquots of 1 ml were withdrawn at intervals and neutralised by addition of

weak acid ion exchange resin (Amberlite IRC-50). The resulting methanolic solution was decanted from the resin and analysed by T.L.C. on silica gel. In this fashion the gradual transformation of annotinine into methyl epiannotinate (72) was monitored. The tranformation was complete in 120 hrs. The reaction mixture was concentrated to dryness *in vacuo*. The residue was dissolved in a mixture of water (10 mls) and chloroform (25 mls). The organic layer was collected and the aqueous layer extracted twice with chloroform (25 mls). The chloroform extracts were combined and dried with $MgSO_4$. Upon concentration *in vacuo* they yielded crude methyl epiannotinate (72). The latter was purified to give pure methyl epiannotinate (72) (22 mgms, 70%).

M.P. 160-1°C.

Treatment of annotinine with sodium methoxide at high temperatures

If the previous sequence for the preparation of methyl epiannotinate was repeated, with the added factor that the reaction solution was refluxed, then the annotinine is consumed faster and upon work up the product is a mixture of two compounds. These can be separated by differential crystallization from ether, whereupon annotinine hydrate (75) is obtained in hard, clear, rhombs.

M.P. 230-2°C (9 mgms, 30%). Concentration of the ether mother liquor *in vacuo*, and dissolution of the residue in methanol gave upon crystallization, methyl epiannotinate (72) (12 mgms, 38%).

Preparation of ester "M" (73)

Freshly cut potassium (31 mgms, 0.8 mmoles) was dissolved in dry methanol (30 mls). When the solution had cooled to room temperature annotinine (28 mgms, 0.09 mmoles) was added. The mixture was stirred at room temperature for 150 hours. Analysis by T.L.C. at this point indicated the annotinine had been completely converted to methyl epiannotinate. The solution was concentrated *in vacuo* for 30 min. Upon cooling, the residue was treated with water (20 mls) whereupon a white precipitate formed. This was collected by filtration, washed with water and methanol and dried *in vacuo* to give ester "M" (73) (21.3 mgms, 76%). M.P. 298°C.

P.M.R. ($CDCl_3/CD_3CO_2D$): δ 4.0 (b, 1H, C<u>H</u>—OH, C-5), δ 3.82 (m, 1H, C<u>H</u>—OH, C-10), δ 3.67 (s, 3H, $CO_2-C\underline{H}_3$) δ 3.56-2.86 (6H), δ 2.65-2.30 (3H), δ 2.0-1.3 (6H), δ 0.97 (d, 3H, $CH(C\underline{H}_3)$).

I.R. (nujol): 3370 cm^{-1}, (s, —OH), 1719 cm^{-1} (s, $-CO_2Me$).

Mass spectrum: 307 (M^+, $C_{17}H_{25}NO_4$, 21%), 248 ($C_{15}H_{22}NO_2$, 100%), 166 (22%).

Preparation of ester "M" (73) from methyl epiannotinate (72)

Repetition of the previous sequence with the substitution of methyl epiannotinate (72) (29 mgms, 0.09 mmoles) for annotinine, followed by immediate work up using the same procedure gave ester "M" (20.8 mgms, 71%).

Preparation of ester "M" (73) from annotinine and rubidium methoxide

The following procedure was performed in a glove box. Rubidium metal (634 mgms, 7.42 mmoles) was melted and poured into a flask containing dry methanol (30 mls, dried by distillation from magnesium). A violent reaction ensued. When the methanolic solution had cooled to room temperature annotinine (71) (250 mgms, 0.81 mmoles) was added and the reaction mixture stirred at room temperature for 72 hrs. At this point T.L.C. analysis indicated complete conversion to methyl epiannotinate (72). The reaction solution was concentrated *in vacuo* and the residue heated at 60°C for 30 mins. *in vacuo*. Upon cooling, the residue was treated with water (20 mls), whereupon a white precipitate formed. This was collected by filtration, washed with water and methanol and dried *in vacuo* to give ester "M" (73). Yield 75 mgms (29%).

Preparation of methyl anhydroepiannotinate (87)

To a solution of methyl epiannotinate (72) (36 mgms, 0.11 mmoles) in methylene chloride (30 mls) was added pyridine (26 mgms, 0.33 mmoles) and thionyl chloride (20 mgms, 12.25 µl, 0.165 mmoles). The mixture was stirred at room temperature for 4 hours. Analysis by T.L.C. on silica gel indicated complete conversion of the starting material into a new, less polar compound. The reaction mixture was washed with saturated $NaHCO_3$ solution and water and dried over $MgSO_4$. Concentration of the organic phase *in vacuo* followed by treatment of the residue under high vacuum overnight to remove traces of pyridine gave a residue which was identified as the desired product, methyl anhydroepiannotinate (87).
Yield 28.7 mgms (86%).

I.R. ($CHCl_3$): 1737 cm^{-1} (s, CO_2Me).

P.M.R. ($CDCl_3$): δ 5.66 (m, 1H, C=C<u>H</u>, C-5), δ 3.71 (s, 3H, $CO_2-C\underline{H}_3$), δ 4.0-1.3 (m, 16H), δ 1.03 (d, 3H, $CH-C\underline{H}_3$).

Mass spectrum: 289 (M^+, 25%), 202 ($C_{13}H_{16}NO$, 24%), 188 ($C_{12}H_{14}NO$, 100%), 160 ($C_{10}H_{10}NO$, 50%), 158 (38%), 132 ($C_9H_{10}N$, 20%), 130 (22%).

Attempted hydrogenation of methyl anhydroepiannotinate (87)

Platinum oxide (5 mgms) was added to a solution of methyl anhydroepiannotinate (87) (10 mgms, 0.034 mmoles) in methanol (25 mls). The mixture was treated with hydrogen at 40 p.s.i. and 70°C with continuous shaking on a Parr Hydrogenation apparatus for 4 days. Examination of the reaction mixture at this point indicated only the presence of the starting material. The latter was recovered by filtration to remove the platinum black and concentration of the filtrate *in vacuo* to give the starting material.

Attempted hydrogenation of methyl anhydroepiannotinate (87) in acetic acid

A mixture of methyl anhydroepiannotinate (87) (10 mgms, 0.034 mmoles) and platinum oxide (5 mgms) in acetic acid (20 mls) was treated with hydrogen at 40 p.s.i. and continuous shaking for 2 days. Examination of the reaction mixture by T.L.C. on silica gel indicated the presence of a new compound in addition to the starting material. The hydrogenation was continued after 1 drop of perchloric acid was added. Within 1 day the reaction mixture consisted solely of the new compound. This was recovered by filtration and concentration of the filtrate *in vacuo*. Yield 7.6 mgms (80%). This compound has a remarkable tendency to sublime; even at room temperature it slowly creeps up the side of a vial. This enables

it to be purified with ease. M.P. 155-158°C.

I.R. ($CHCl_3$, 0.5 mms): 3450 cm^{-1}, (broad, OH); 1785 cm^{-1} (strong, five-membered lactone).

P.M.R. ($CDCl_3$): δ 5.5 (d, 1H, C<u>H</u>=C, J = 5.8), δ 4.1 (m, 3H, C<u>H</u>—OH, C<u>H</u>—O—CO), δ 3.5-1.2 (m, 14H), δ 1.1 (d, 3H, CH—C<u>H</u>$_3$).

Mass spectrum: 275 (M^+, 90%), 232 (10%), 204 (60%), 189 (80%), 172 (100%).

<u>Attempted reaction of methyl anhydroepiannotinate (87) and hydrogen sulphide</u>

A solution of methyl anhydroepiannotinate (87) (5 mgms, 0.017 mmoles) and azobisisobutyl nitrile (1 mgm) in benzene (20 mls) was saturated with hydrogen sulphide gas at room temperature. This solution was refluxed for 24 hrs. using a rubber balloon to retain the hydrogen sulphide. At this point, analysis by T.L.C. indicated no compounds other than starting materials in the reaction mixture. The reaction flask (pyrex) was then irradiated with a 140 W. Hanovia low pressure mercury U.V. lamp and refluxed for 48 hours. Analysis by T.L.C. again indicated that no reaction had occured. The starting material was recovered intact by chromatographic separation of the azobisisobutylnitrile on silica gel.

Preparation of the mesylate (89)

Mesyl chloride (12.0 mgms, 8.6 µl, 0.11 mmoles) was added to a solution of methyl anhydroepiannotinate (87) (23 mgms, 0.071 mmoles) and pyridine (11 mgms, 0.14 mmoles) in dry methylene chloride (30 mls). The solution was stirred at room temperature for 4 hours and then mixed with 50 mls of ice cold saturated $NaHCO_3$ solution. The organic layer was removed and the aqueous phase extracted twice more with methylene chloride. The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo* to give the mesylate (89) as a pale yellow oil. Yield 22 mgms (80%).

P.M.R. ($CDCl_3$): δ 4.90 (m, 1H, C<u>H</u>—OMes), δ 3.70 (s, 3H, CO—C<u>H</u>$_3$), δ 3.01 (s, 3<u>H</u>, O—SO_2—C<u>H</u>$_3$), δ 1.13 (d, 3H, CH—C<u>H</u>$_3$).

Mass spectrum: chemical ionization with ammonia; 386 (M+1, <1%), 326 (8%), 308 (10%), 290 (100%).

Attempted reduction of mesylate (89)

Sodium cyanoborohydride (12.6 mgms, 0.2 mmoles) was added to a solution of mesylate (89) (20 mgms, 0.052 mmoles)

in hexamethylphosphoramide (20 mls). The solution was stirred at room temperature for 24 hours. Examination of the reaction mixture by T.L.C. on silica gel indicated only the presence of the starting material. The reaction mixture was heated to 75°C and stirred for 24 hours. Upon examination by T.L.C. on silica gel a new compound was detected in addition to the starting material. Comparison of the R.f. of this material with that of the olefin (87) implied that they were identical. The reaction mixture was worked up by diluting it with water (100 mls) and extracting this aqueous solution with methylene chloride (3 x 30 mls). The organic extracts were combined and dried over $MgSO_4$ and concentrated *in vacuo*. The residue upon examination by P.M.R. indicated the presence of the olefin (87) by the distinctive olefinic absorption at δ 5.5 in that compound.

Attempted reduction of the mesylate (89) using sodium cyanoborohydride and 18-crown-6

The previous experiment was repeated with the addition of 18-crown-6 (.5 mgms, 0.002 mmoles, 0.01 equivalents) to the reaction mixture. At room temperature the results were identical. At higher temperatures (75°C) there was also production of the olefin (87). Increasing the amount of 18-crown-6 ten times (5 mgms, 0.02 mmoles, 0.1 equivalents) was without effect. These experiments were

repeated using dimethoxyethane (30 mls) as solvent instead of hexamethylphosphoramide. The results however were the same, in that only starting material could be recovered.

Treatment of mesylate (89) with lithium iodide

Lithium iodide (56 mgms, 0.42 mmoles) was added to a solution of the mesylate (89) (10 mgms, 0.04 mmoles) in dry acetonitrile (20 mls). The solution was stirred at room temperature for 24 hours. Analysis of the reaction mixture by T.L.C. on silica gel indicated the presence of a small amount of the olefin (87). The experiment was repeated with addition of 1 drop of triple distilled mercury.[78] After 24 hours no olefin could be detected, but the starting material remained the only component of the reaction mixture.

Attempted Jones' Oxidation of methyl epiannotinate (72)

Jones' reagent (8 M aqueous sulphuric acid) was added drop by drop to a solution of methyl epiannotinate (5 mgms, 0.016 mmoles) in acetone (5 mls) until the orange colour was sustained. After stirring for 5 mins. the reaction was quenched by the addition of 4 drops of isopropyl alcohol. The acetone solution was decanted from the polymeric chromium, diluted with an excess of methylene chloride and washed with saturated $NaHCO_3$ solution. The residue, obtained after drying with Na_2SO_4 and concentration

in vacuo, was shown to be an equal mixture of two compounds. The I.R. spectrum shows a five-membered lactone carbonyl at 1780, an ester carbonyl at 1730 and a ketone carbonyl at 1710. On this basis the structures (90) and (91) were assigned to these two compounds.

Attempted 2-phase Jones' Oxidation of methyl epiannotinate (72)

An aqueous solution of chromic acid (3 mls of 0.01 M) was added to a solution of methyl epiannotinate (72) (10 mgms, 0.03 mmole) in benzene (3 mls). The solution was stirred at room temperature for 10 minutes and then quenched by the addition of 4 drops of isopropyl alcohol. The reaction mixture was diluted with methylene chloride (10 mls) and washed with saturated $NaHCO_3$ and dried over Na_2SO_4. Concentration of this solution *in vacuo* gave a residue which on T.L.C. analysis with silica gel indicated the presence of the two ketones (90) and (91). This prognosis was confirmed by I.R.

Attempted oxidation of methyl epiannotinate (72) using Collin's Reagent

A solution of chromic anhydride (20 mgms, 0.2 mmoles) and pyridine (31.6 mgms, 33 µl, 0.4 mmoles) in methylene chloride (10 mls) was prepared. This solution was stirred for 10 minutes and then methyl epiannotinate (16 mgms,

0.05 mmoles) was added. The mixture was stirred for 10 minutes at room temperature and then worked up by passing the solution through a small column of alumina. The eluate was concentrated *in vacuo* to give a residue. This was identified as the lactam (92) by its I.R. spectrum.

I.R. ($CHCl_3$, 0.5 mms): 1735 cm^{-1} (s, CO_2CH_3), 1710 cm^{-1} (s, R_2CO), 1650 cm^{-1} (s, —CO—N).

Oxidation of methyl epiannotinate using N-chlorosuccinimide/ dimethyl sulphide

Dimethyl sulphide (30 µl, 0.41 mmoles) was added to a stirred solution of N-chlorosuccinimide (40 mgms, 0.3 mmoles) in toluene (10 mls) at 0°C. A white precipitate appeared immediately. The solution was cooled to -25°C (carbon tetrachloride/dry ice cryostat) and methyl epiannotinate (67 mgms, 0.2 mmoles) in toluene (5 mls) was added. The mixture was stirred at -25°C for 2 hours and then a solution of triethylamine (30 mgms, 40 µl, 0.3 mmoles) in toluene (0.5 mls) was added. The reaction mixture was warmed to room temperature and diluted with methylene chloride (30 mls). This solution was extracted once with pH 4 buffer (50 mls, Fischer 1M) and four times with warm water (40 mls). The organic phase was dried with $MgSO_4$ and concentrated *in vacuo* to give the desired ketone (94). Yield 60 mgms (91%). The ketone could be recrystallized from ether to colourless rhombs. M.P. 161-162°C.

I.R. ($CHCl_3$, 0.5 mms): 1731 cm^{-1} (S, CO_2CH_3), 1709 cm^{-1} (s, R_2CO).

Mass spectrum: 305 (M^+, $C_{17}H_{23}NO_4$), 263 ($C_{14}H_{17}NO_4$, 12%), 234 ($C_{13}H_{16}NO_3$, 14%), 204 ($C_{12}H_{14}NO_2$, 100%), 176 ($C_{11}H_{14}NO$, 20%).

P.M.R. ($CDCl_3$): δ 3.7 (s, 3H, $CO_2-C\underline{H}_3$); δ 1.1 (d, 3H, $CH-C\underline{H}_3$).

Reaction of ketone (94) with tosylhydrazide

Tosylhydrazide (30 mgms, 0.16 mmoles) was added to a solution of ketone (94) (10 mgms, 0.033 mmoles) in toluene. The solution was refluxed for 48 hours. Examination of the reaction mixture by T.L.C. on silica gel indicated that the starting material had been converted into a new, more polar compound. Removal of the toluene under reduced pressure followed by prepratory T.L.C. on silica gel plates (1.0 mm layer) allowed this compound to be recovered. It was identified as the acyl tosyl hydrazide (95). Yield 3 mgms (20%).

I.R. ($CHCl_3$, 0.5 mms): 1710 cm^{-1} (s, R_2CO), 1690 (sh, m, $-CO-NH-$), 1600 (m, aromatic ring).

P.M.R. ($CDCl_3$): δ 7.9-7.2 (m, 4H), δ 2.4 (s, 3H, CH_3-Ar), δ 1.05 (d, 3H, $CH-C\underline{H}_3$)

Mass spectrum: 459 (M^+, $C_{23}H_{29}N_3O_5S$, 4%), 304 ($C_{16}H_{23}N_3O_3$, 10%), 262 ($C_{13}H_{16}N_3O_3$, 40%), 204 ($C_{12}H_{14}NO_2$, 100%), 188 ($C_{12}H_{14}NO$, 18%).

Attempted reaction between methyl epiannotinate (72) and methyl iodide

Methyl iodide (24 mgms, 10 µl, 0.17 mmoles) was added to a solution of methyl epiannotinate (72) (10 mgms, 0.03 mmoles) and pyridine (14.2 mgms, 14.5 µl, 0.18 mmoles) in methylene chloride. The mixture was stirred at room temperature for 24 hours. Analysis by T.L.C. on silica gel at this point indicated only the presence of starting material. The solution was refluxed for 24 hours and upon examination again by T.L.C. contained only the starting material.

Attempted reaction between methyl epiannotinate (72) and methyl iodide using sodium hydride

Sodium hydride (50% in oil, 2 mgms, 0.042 mmoles) was added to a solution of methyl epiannotinate (72) (10 mgms, 0.033 mmoles) and methyl iodide (24 mgms, 10 µl, 0.17 mmoles in benzene (20 mls). The suspension was stirred at room temperature for 8 hours. After this period of time, the majority of the sodium hydride had been consumed and examination of the reaction mixture by T.L.C. on silica gel indicated the presence of a new

component, less polar than the starting material. This new component was identified as annotinine by comparison with an authentic sample on T.L.C. The I.R. spectrum of the reaction mixture also showed the characteristic five-membered lactone of annotinine at 1780 cm^{-1}.

Attempted reaction between methyl epiannotinate (72) and chloromethylmethyl ether using sodium hydride

The previous experiment was repeated exactly with the substitution of chloromethyl methylether (13.7 mgms, 12.9 µl, 0.17 mmoles) for the methyl iodide. The result was the same, that is, a mixture of methyl epiannotinate (72) and annotinine (71).

Attempted reaction between methyl epiannotinate (72) and dimethyl t-butylsilyl chloride and imidazole

Dimethyl t-butylsilyl chloride (7.4 mgms, 0.05 mmoles) was added to a solution of methyl epiannotinate (72) (10 mgms, 0.033 mmoles) and imidazole (6.6 mgms, 0.1 mmoles) in dimethylformamide (10 mls). The solution was stirred at 35°C for 24 hours. Examination of the reaction mixture at this point indicated only the presence of starting material. The reaction temperature was increased to 80°C. After 12 hours, examination by T.L.C. indicated the production of a new component, which was however quickly identified as annotinine (71).

Preparation of the axial monotrifluoroacetate (96)

Trifluoroacetic anhydride (16.8 mgms, 11.25 µl, 0.08 mmoles) was added to a suspension of ester "M" (73) (6 mgms, 0.02 mmoles) in pyridine (8 mgms, 8.2 µl, 0.1 mmoles) in methylene chloride (4 mls). The mixture was stirred at room temperature overnight and then diluted with methanol (20 mls). This solution was passed through a column of IRC-50 (about 20 gms) that had been thoroughly flushed with methanol (to remove water). The eluate was concentrated under reduced pressure and the residue exposed to high vacuum to remove traces of pyridine. This residue was identified as the axial mono trifluoroacetate (96). Yield 5.2 mgms (60%).

I.R. ($CHCl_3$, 0.5 mms): 3590 cm^{-1} (m, OH), 1785 cm^{-1} (s, CF_3CO-O), 1718 cm^{-1} (s, CO_2-CH_3), 1660 cm^{-1} (s),

P.M.R. ($CDCl_3$): δ 5.0 (b.s., 1H, $CH-O-COCF_3$), δ 4.5 (m, 1H, $\underline{C}H-OH$), δ 3.6 (s, 3H, $CO-O-CH_3$), δ 0.91 (d, 3H, $CH-C\underline{H}_3$).

Mass spectrum: 403 (M^+, $C_{19}H_{24}NO_5F_3$, 16%), 344 ($C_{17}H_{21}NO_3F_3$, 54%), 307 ($C_{17}H_{25}NO_4$), 302 ($C_{14}H_{15}NO_3F_3$, 20%), 290 ($C_{17}H_{24}NO_3$, 33%), 248 ($C_{14}H_{18}NO$, 100%), 230 ($C_{14}H_{16}NO_2$, 15% and $C_{15}H_{20}NO$, 33%), 206 ($C_{12}H_{16}NO_2$, 58%), 198 ($C_{12}H_{14}NO$).

Conversion of the axial trifluoroacetate (96) to ester "M" (71)

A solution of the axial trifluoroacetate (96) (5 mgms, 0.01 mmoles) in methanol (1 mls) was treated with 2 drops of diluted $NaHCO_3$ solution. Ester "M" started to precipitate almost immediately. It was collected by filtration and identified *via* its M.P.

Preparation of the equatorial monotrichloro acetate (97)

Trichloroacetyl chloride (18.2 mgms, 11.2 µl, 0.1 mmoles) was added to a suspension of ester "M" (12.3 mgms, 0.04 mmoles) in methylene chloride (5 mls) and pyridine (12 mgms, 0.15 mmoles). The mixture was stirred for 1 hour at room temperature by which time the ester "M" had dissolved. The reaction was quenched by mixing with an equal volume of ice-cold saturated $NaHCO_3$ solution. The methylene chloride layer was drawn off and the aqueous phase extracted twice more with methylene chloride (2 x 5 mls). Drying of the methylene chloride layers and concentration under reduced pressure gave a residue which on treatment with high vacuum (to remove pyridine) afforded the mono trichloroacetate (97) as the sole product. Yield 16.4 mgms (91%).

P.M.R. ($CDCl_3$): δ 4.9 (d of t, 1H, J = 8, 8, 4, C<u>H</u>—$OCOCl_3$);

δ 3.95 (b.s., 1H, C<u>H</u>—OH), δ 3.7 (s, 3H, $-CO_2C\underline{H}_3$), δ 3.4 - 1.3 (m, 16H), δ 0.96 (d, 3H, C<u>H</u>—CH_3).

I.R. ($CHCl_3$, 0.5 mms): 1765 cm^{-1} (s, $-CO-CCl_3$), 1718 cm^{-1} (s, $-CO_2-CH_3$).

Mass spectrum: 453 ($M^+ + 2$, 10%), 451 (M^+, 10%), 394 (18%), 392 (18%), 290 (30%), 230 (35%), 222 (37%), 149 (100%).

<u>Preparation of the ditrichloroacetate (98) from ester "M" (71)</u>

Trichloroacetyl chloride (40 mgms, 24.5 ml, 0.22 mmoles) was added to a suspension of ester "M" (12 mgms, 0.04 mmoles) in methylene chloride (5 mls) and pyridine (40 mgms, 0.5 mmoles). The mixture was stirred for 24 hours at room temperature. It was then mixed with an equal volume of ice-cold saturated $NaHCO_3$ solution. The organic layer was drawn off and the aqueous phase extracted twice more with methylene chloride. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was treated with high vacuum at room temperature (to remove pyridine) to give the ditrichloroacetate (98). Yield 21.3 mgms (90%).

P.M.R. ($CDCl_3$): δ 5.15 (b.s., 1H, C<u>H</u>—O—$COCl_3$), δ 3.84 (d of t, 1H, J = 8, 8, 4.5, C<u>H</u>—O—$COCCl_3$), δ 1.68 (s, 3H, CO—OCH_3), δ 0.98 (d, 3H, CH—$C\underline{H}_3$).

I.R. ($CHCl_3$, 0.5 mms): 1768 cm^{-1} (v.s., $CO-CCl_3$).

Preparation of the axial monotrichloroacetate (99) from the ditrichloroacetate (98)

A saturated solution of $NaHCO_3$ in water (10 mls) was stirred vigorously with a solution of the axial monotrichloroacetate (99) (9 mgms, 0.02 mmoles) in methylene chloride for 24 hours. Examination of the organic layer by T.L.C. on silica gel indicated that the starting material had been entirely converted into a new more polar compound. The organic layer was separted off, washed with water and dried over $MgSO_4$. On concentration under reduced pressure it yielded a residue, which was identified as the axial monotrichloroacetate (99). Yield 6 mgms (88%).

P.M.R. ($CDCl_3$): δ 5.12 (b.s., 1H, $C\underline{H}-O-CO-CCl_3$), δ 3.8 (m(?), 1H(?), $C\underline{H}-OH$), δ 3.75 (s, 3H, $CO_2-C\underline{H}_3$), δ 1.0 (d, 3H, $C\underline{H}-CH_3$).

I.R. ($CHCl_3$, 0.5 mms): 3350 cm^{-1} (b, m, —OH), 1765 cm^{-1} (s, —CO—CCl_3), 1720 cm^{-1} (s, CO_2—CH_3), 1660 cm^{-1} (s).

Mass spectrum: 455 ($C_{19}H_{24}NO_5Cl_3$, 4%), 453 ($C_{19}H_{24}NO_5Cl_3$, 13.5%), 451 (M^+, $C_{19}H_{24}NO_5Cl_3$, 14%), 394 (30%), 392 (27%), 352 ($C_{14}H_{15}NO_3Cl_3$, 6%), 350 ($C_{14}H_{15}NO_3Cl_3$, 7%), 290 ($C_{17}H_{24}NO_3$, 100%), 230 ($C_{15}H_{20}NO$, 50% and $C_{14}H_{16}NO_2$, 23%), 170 ($C_{12}H_{12}N$, 34%).

Preparation of the trichloroacetoxy ketone (100)

Dimethyl sulphide (30 µl, 0.41 mmoles) was added to a stirred solution of N-chlorosuccimide (40 mgms, 0.3 mmoles) in toluene. A white precipitate appeared immediately. The solution was cooled to -25°C (carbon tetrachloride/dry ice) and the trichloroacetate (97) (90.2 mgms, 0.2 mmoles) in toluene (5 mls) was added. The mixture was stirred at -25°C for 2 hours and then a solution of triethylamine (30 mgms, 40 µl, 0.3 mmoles) in toluene 0.5 mls) was added. The reaction mixture was warmed to room temperature and diluted with methylene chloride (30 mls). This solution was extracted once with pH 4 buffer (50 mls, Fischer 1M) and four times with water (40 mls). The organic phase was dried with $MgSO_4$ and concentrated *in vacuo* to give the desired ketone (100). Yield 69 mgms (76%).

I.R. ($CHCl_3$, 0.5 mms): 1770 cm^{-1} (s, $CO-CCl_3$), 1721 cm^{-1} (s, $-CO_2-CH_3$), 1710 (sh, R_2CO), 1240 cm^{-1} (s).

P.M.R. ($CDCl_3$): δ 4.81 (m, 1H, $C\underline{H}-O-COCCl_3$), δ 3.73 (s, 3H, $-CO_2-CH_3$) δ 1.16 (d, 3H, $C\underline{H}-CH_3$).

Mass spectrum: 451 (M + 2, 52%), 449 (M, 52%), 436 (40%), 434 (40%), 402 (7%), 400 (7%), 290 (15%), 288 (10%), 272 (12%), 246 (100%), 178 (16%).

Preparation of the hydroxy ketone (101)

A mixture of saturated aqueous $NaHCO_3$ solution (30 mls) and trichloroacetoxy ketone (100) (68 mgms, 0.2 mmoles) in methylene chloride (30 mls) was vigorously stirred for 24 hours at room temperature. The organic layer was then drawn off, washed with water, dried over $MgSO_4$, and concentrated *in vacuo* to yield the hydroxy ketone (101). Yield 41 mgms (90%).

P.M.R. ($CDCl_3$ + 1 drop CD_3CO_2D): δ 4.0 (t of d, 1H, J = 8, 8, 2.5, $C\underline{H}-O-COCCl_3$), δ 3.70 (s, 3H, $CO_2-C\underline{H}_3$), δ 1.08 (d, 3H, $CH-C\underline{H}_3$).

I.R. ($CHCl_3$, 0.5 mms): 1715 cm^{-1} (v.s., R_2CO, CO_2-CH_3).

Mass spectrum: 305 ($C_{17}H_{23}NO_4$, 17%), 290 ($C_{16}H_{20}NO_4$, 100%), 246 ($C_{14}H_{16}NO_3$, 62%), 218 ($C_{13}H_{16}NO_2$, 31%).

Reduction of the ketone (101) using lithium in ammonia

Freshly cleaned lithium metal was extruded using a sodium press to form very thin wire. This wire was quickly weighed to determine its weight to length ratio. In this fashion the wire was found to weigh 0.14 mgms/cm. A three neck flask was fitted with a serum cap, a stopper and a dry-ice condenser. Freshly distilled ammonia (from sodium) (10 mls) was introduced, followed by lithium wire (96 mm long, 1.32 mgms, 0.19 mmoles) and the mixture was stirred for 10 minutes. Then the ketone (101) (10 mgms, 0.032 mmoles) in dry T.H.F. (10 mls) containing t-butanol (1 drop) was added. The mixture was stirred for 30 minutes during which time the ammonia was allowed to reflux. The reaction was quenched by the addition of ammonium chloride (*circa* 0.5 gms) and the ammonia was allowed to evaporate. The residual T.H.F. solution was diluted with ether (20 mls) and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give a residue which was identified as the diol (103). Yield 2.4 mgms (24%).

P.M.R. ($CDCl_3$): δ 3.9 (m, 2H, C<u>H</u>—OH), δ 3.64 (s, 3H, CO—O—C<u>H</u>$_3$), δ 1.12 (d, 3H, CH—C<u>H</u>$_3$).

on addition of 1 drop of trichloroacetylisocyanate the multiplet at δ 3.9 moves to δ 5.2.

I.R. ($CHCl_3$, 0.5 mms): 3510 cm^{-1} (m, —OH), 3400 cm^{-1} (m, —OH), 1735 cm^{-1} (s, b, CO_2CH_3), 1120 cm^{-1} (s, C—OH), 1095 cm^{-1} (s, C—OH).

Mass spectrum: 309 (M^+, $C_{17}H_{27}NO_4$, <1%), 308 ($C_{17}H_{26}NO_4$, 6%), 267 ($C_{14}H_{21}NO_4$, 16%), 209 ($C_{12}H_{19}NO_2$, 14%), 208 ($C_{12}H_{18}NO_2$, 100%), 190 ($C_{12}H_{16}NO$, 30%), 172 ($C_{12}H_{14}N$, 4%).

Preparation of D_4-methyl epiannotinate

Sodium metal (0.2 gms) was added to D_4-methanol (5 mls) and the solution was cooled to room temperature. Methyl epiannotinate (25 mgms, 0.08 mmoles) was added and the solution was stirred for 2 days. The methanol was removed using reduced pressure and the residue taken up in a mixture of water (10 mls) and methylene chloride (10 mls). The methylene chloride solution was dried over $MgSO_4$ and concentrated *in vacuo* to yield the product. Yield 23 mgms (92%). The product was found to be a 50/50 mixture of D_3 and D_4 methyl epiannotinate.

Mass spectrum:

A) Ionization with ammonia: 312 (D_4, 100%), 311 (D_3, 100%).

B) Electron bombardment: 312 (D_4, <1%), 311 (D_3, <1%), 209 (21%), 268 (23%), 240 (13%), 239 (12%), 208 (17%), 207 (100%), 206 (88%), 190 (65%).

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THE STRUCTURE OF CYATHIC ACID

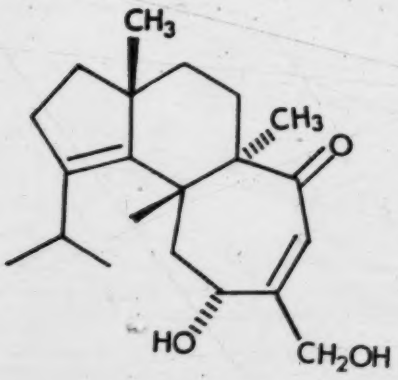
CHAPTER THREE

INTRODUCTION

The search for new and useful compounds has led the natural product chemist to investigate more and more of the myriad life forms of this planet. Given the vast number of such life forms and the fact that the majority of these have yet to be examined, it is only after some deliberate thought that the decision to open up a new frontier can be made. Indeed deliberate thought is often not sufficient to guarantee success in such a venture. Some clue, some beckoning sign is a necessary adjunct for any decision. Thus it was a report of bacteriostasis in the distant realms of the *Nidulariaceae*[96] that prompted an investigation of this life form.

The *Nidulariaceae* constitute a family of basidiomycetic fungi commonly known as the bird's nest fungi.[97] They are widely distributed, some species having been found in most countries. A typical member of bird's nest fungi is composed of two parts. Beneath the ground are large arrays of *mycelia* while above the ground a fruiting body or *peridium* is apparent. It is the latter which gives these fungi their common name. The *peridium* is cup shaped and often filled with lenticular bodies called *peridioles*. Thus it bears a general resemblance to a miniature bird's nest containing eggs.

Initially, bacteriostasis was observed in a new member of this family, *Cyathus helenae*, but subsequently was also reported for *C. striatus*, *C. lionbatus* and *C. poeppigii*.[96] Work undertaken in these laboratories to determine the source of the anti-bacterial activity of *Cyathus helenae* led to the discovery of a new class of diterpenes[14] - the cyathins. Typical of this new class were cyathin A_3 (19) and allocyathin B_3 (107).



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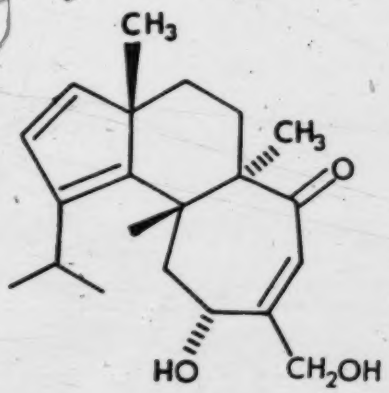
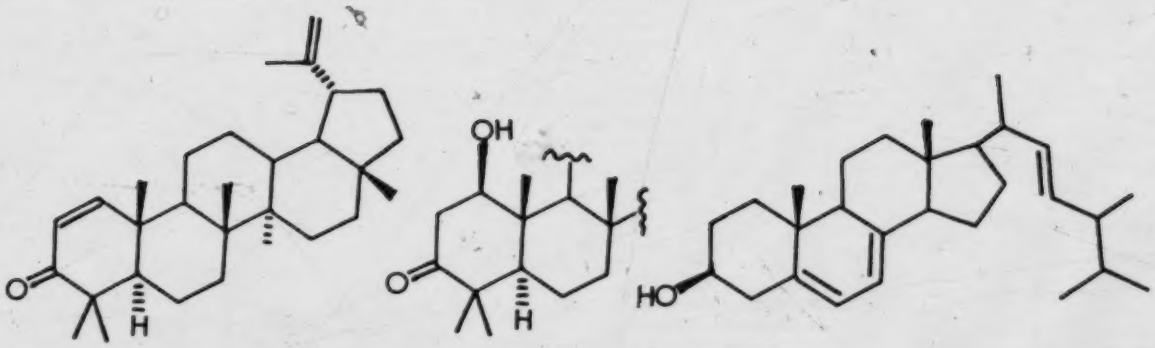


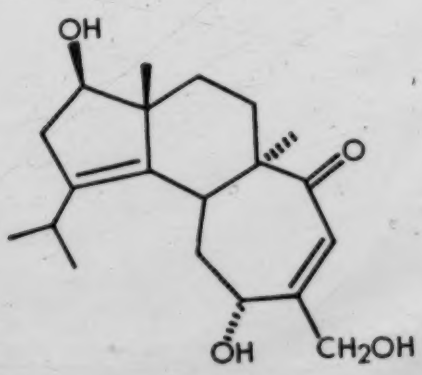
(107)

The cyathin diterpenes were recovered from the liquid medium on which *C. helenae* was grown but examination of the mycelia led to the identification of three known natural products. These were glochindone (108), glochidonol (109) and ergosterol (110)[98]



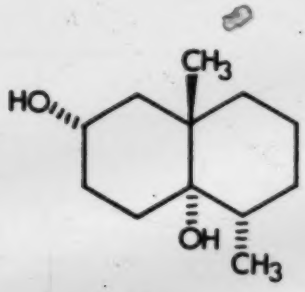
(108) (109) (110)

In a logical step the search for further bacteriostatic compounds was extended to the other members of the *Nidulariacae*. Thus an investigation of *Cyathus africanus* uncovered the presence of further members of the cyathin family of diterpenes.[99] These were named cyafrins. A typical member of the cyafrins is cyafrin A_4 (113)



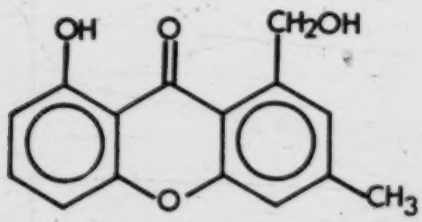
(113)

Examination of the metabolites of *Cyathus bulleri* led to the characterization of cybullol[90] which has the structure shown below (114)



(114)

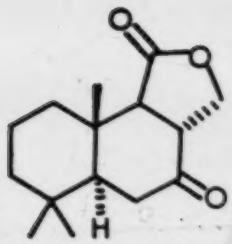
A study of the culture broth of *Cyathus intermedius* disclosed the presence of a new xathone, 1-hydroxy-6-methyl-8-hydroxymethylxanthone[101] (115).



(110)

Another member of *Nidulariacae*, not a *Cyathus* species, *Mycocalia reticulata* was found to produce a series of

sequiterpene lactones (116), (117) and (118).[101]



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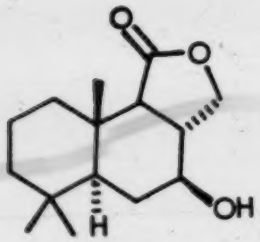

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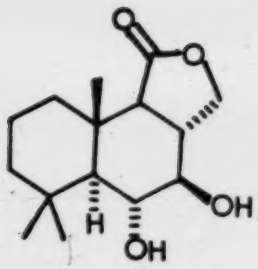
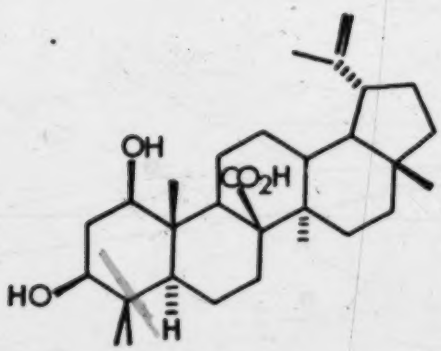


(118)

At this point a report[103] from another group of investigators appeared which concerned the bacteriostatic activity of *Cyathus striatus*. These investigators working with a strain (No. 12) of *C. striatus* found some compounds of a seemingly sesterterpene nature which they called striatins. Although they were unable to determine the structure of these striatins they were able to associate the bacteriostatic activity of *C. striatus* with these compounds.

We possessed at this point a different strain of *C. striatus* known as 68037-II. We decided to look for these striatins. Furthermore the striatins were reportedly recovered in high yield from the mycelia of *C. striatus* but we had observed in an earlier test only weak bacteriostasis in the growth medium and none in the mycelia.

A series of experiments was undertaken to reproduce as closely as possible the conditions and techniques whereby striatins could be recovered from the *C. striatus*. Our earlier observations concerning the presence of bacteriostatic compounds in the growth medium were confirmed and again no such compounds were found in the mycelia. However a thorough investigation of the components of the mycelia uncovered the presence of glochidone (108) and glochidonol (109).. Furthermore a new triterpene acid was also present. This was isolated as a diacetate of the free acid. The parent diol was shown to have the structure (119). This compound we named cyathic acid.



(119)

This, then is an account of the experiments by which we elucidated the structure of cyathic acid.

DISCUSSION AND RESULTS

Cyathus striatus (Strain No. 68037-II) was grown in submerged liquid culture. This involved taking a small quantity of the fungus and inoculating 125 mls of yeast/malt medium (y.m.) under sterile conditions. This initial growth known as the First Generation or G-1 was kept at room temperature and shaken on a rotary shaker for 5 days. By this time, particles of new growth were clearly visible. To increase the growth rate these buds were broken up by the addition of 25 pyrex glass beads and the shaking continued for a further 4 days. This culture was then used to inoculate 4 further flasks containing y.m. medium. In about one week these flasks, known as G-2, were used to inoculate 10 litres of y.m. medium. The latter was connected to a New Brunswick Microferm apparatus and maintained at 22°C for about two weeks. By this time considerable growth has taken place and the fungus was ready for harvesting.

The strain of *C. striatus* used in these experiments was homokaryotic. This means that only one sexual type is present. Thus no fruiting bodies are produced during growth, only mycelia. Indeed the appearance of *C. striatus* grown in submerged culture bears no resemblance whatsoever to the naturally encountered sort. In fact mycelia are generally not observed in nature as they usually *subterrain*.

The upshot of this is that the harvesting of *C. striatus* grown in submerged culture is a task fraught with difficulties. The tangled masses of mycelia are very difficult to remove from the culture medium. Filtration is so slow as to be useless. We solved this problem in two ways. Centrifugation at 1000 x g was generally sufficient to precipitate the mycelia as a jellied mass. However this was a batch process and very time consuming. Alternatively, the mycelia could be precipitated from the culture medium by diluting the latter with an equal volume of ethanol. One disadvantage of this process is that the secondary metabolites found in the mycelia and the medium become mixed.

Initially we chose the centrifugation process and harvested our mycelia in this fashion. The dried mycelia were extracted by boiling the mycelia in a large excess of methanol. Upon concentration the methanol solution yielded approximately 21 gms of material. Not all the extract could be readily redissolved in methanol. This was no doubt due to the retention of moisture by the mycelia so that some water soluble compounds were incorporated into the methanol extract. This problem was overcome by trituration of original material with about 40 mls of boiling methanol. Decanting the methanol and concentration of the latter under reduced pressure gave 4.5 gms of crude extract. This extract represented

the true methanol soluble components of the mycelia.

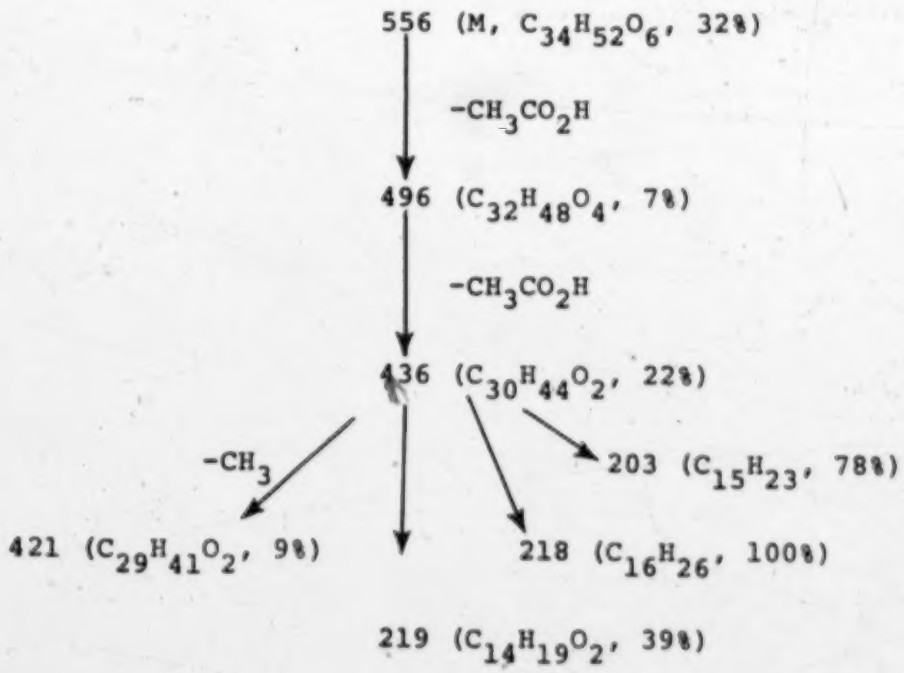
In accordance with the procedure described for the isolation of striatins[103] this methanolic extract was chromatographed on Sephadex LH-20 using a 2.5 x 75 cms column. The eluting agent was methanol and one hundred 5 mls fractions were collected. On standing for a couple of days many of these fractions began to crystallize. However they were first screened for bacteriostatic activity. This was carried out using a standard procedure.[104] Small paper discs impregnated with a small amount of the fraction were placed on a thin layer of *Staphylococcus aureus* growing on Mueller-Hinton medium in petri dishes. These dishes were incubated at 37°C for 24 hours and then examined for signs of bacteriostasis. No such activity was observed.

We then turned our attention to those fractions which obviously contained material and especially those that were crystalline. In this it became apparent that the bulk of the material eluted from the column was confined to three compounds. Thus fractions 51 to 55 yielded 200 mgms of Glochidonol (109). Fractions 38 to 41 were composed for the most part of Glochidone (108). However fractions 43-49 yielded beautifully crystalline material. These fractions were combined and dissolved in 5 mls of methanol. On standing overnight at 4°C white amorphous crystals were deposited. These were

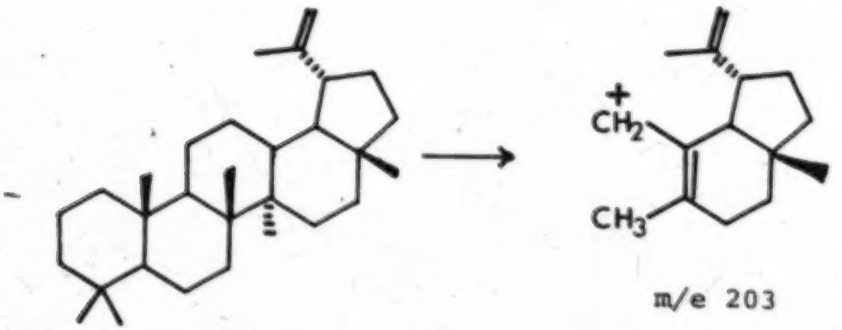
collected by filtration (yield 90 mgms). A futher quantity (60 mgms) was recovered by concentration of the mother liquors. Recrystallization from methanol at room temperature over 8 days yielded beautiful white needles, (yield 150 mgms) which had a m.p. of 311-12°C.

A high resolution mass spectral examination of this compound revealed the molecular formula to be $C_{34}H_{52}O_6$. This formula was confirmed by microanalysis. This information coupled with the m.p. allowed us to confirm that this was a new compound. Certain properties of the mass spectrum implied that the compound was the diacetate of a carboxylic acid and so we named it O,O-diacetylcyathic acid. This prognosis was later confirmed by other evidence.

A detailed interpretation of the mass spectrum of O,O-diacetylcyathic acid is as follows:

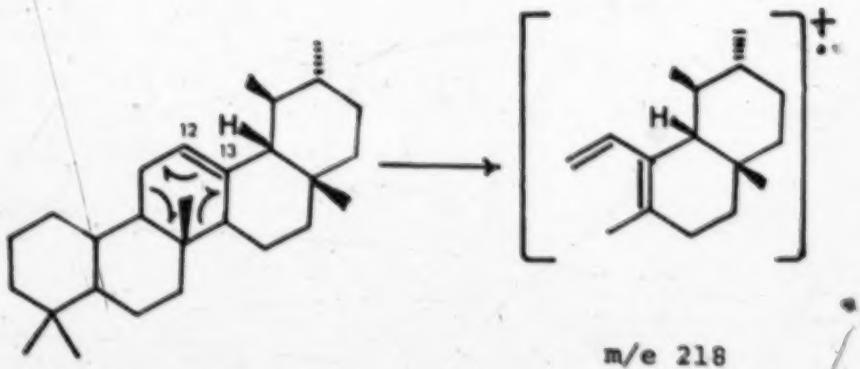


The fragment found at 203 is the first piece of evidence that we are in fact dealing with a triterpene. This is a commonly found fragment in many terpenes.[105] In pentacyclic triterpenes it represents the D and E ring.[106]



m/e 203

The fragment at 218 is a homologue of the 203 fragment but besides that it is not commonly found in triterpenes. In oleananes it equals the 203 in magnitude[105] and represents the C, D and E rings.



m/e 218

This fragmentation is therefore caused by $\Delta^{12.13}$ bond in oleananes. This accounts for its absence in saturated triterpenes.

The fragment found at 219 must therefore be due to the other half of the 436 fragment after the cleavage of the 218 fragment. Thus if the cyathic acid were a pentacyclic triterpene the 219 fragment would represent the A and B rings. Furthermore the absence of any added unsaturations in the C, D and E ring fragments implies that all the oxygens are situated initially in the A and B rings.

The I.R. spectrum of O,O-diacetylcyathic acid shows a strongly H-bonded hydroxy group between 3300 cm^{-1} and 3100 cm^{-1}. A strong carbonyl band occurs at 1741 cm^{-1}. This band coupled with an equally strong doublet at 1248 cm^{-1} implies the presence of acetyl group(s). Another carbonyl band of lesser intensity appears at 1685 cm^{-1}.

Some light was cast on the identity of the carbonyl at 1685 cm^{-1} by examining the U.V. spectrum. If this band is due to an $\alpha\beta$ unsaturated carbonyl this should be immediately apparent from the U.V. spectrum. The latter when measured showed a continuum rising to maximum at 210 n.m. with an extinction coefficient of about 100. Thus an $\alpha\beta$ unsaturated ketone is out of the question. The O.R.D. shows a positive Cotton effect with a maximum at about 210 n.m. This is in keeping with a carboxylic

acid or a derivative thereof.[107]

The P.M.R. spectrum clearly indicates the presence of two acetyl functions as sharp singlets at δ 1.97 and δ 2.0. About δ 4.6 a complex multiplet appears containing 4 hydrogens. A vinylic methyl group appears at δ 1.67 and five aliphatic methyl groups occur as singlets between δ 0.71 and δ 1.0.

At this point some assumptions were made;

A) that the molecule was a lupane triterpene

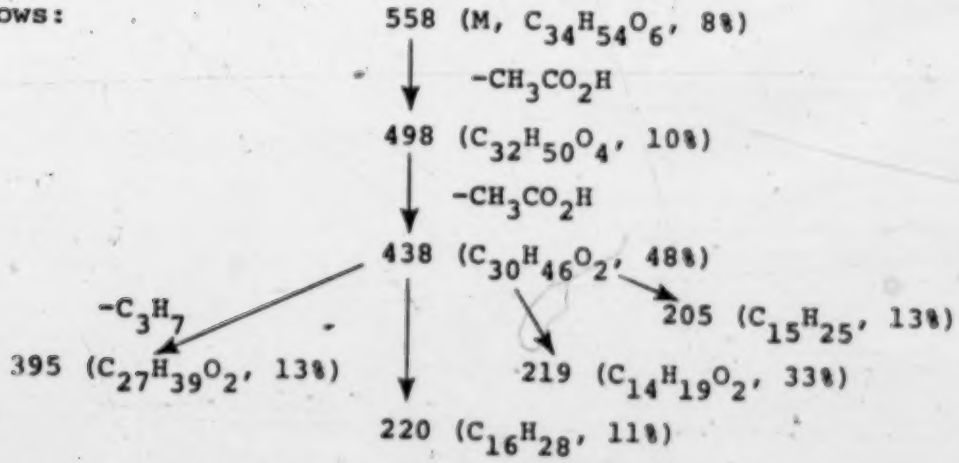
B) that it contained two acetyl groups

C) that the carbonyl function at 1685 cm^{-1} was a carboxyl group.

These suppositions were confirmed in the following series of experiments.

O,O-diacetylcyathic acid was hydrogenated in methanol using Adam's catalyst (PtO_2) to give O,O-diacetyldihydrocyathic acid in 84% yield. The mass spectrum of this compound confirms the molecular formula as $C_{34}H_{54}O_6$.

A detailed interpretation of the mass spectrum is as follows:

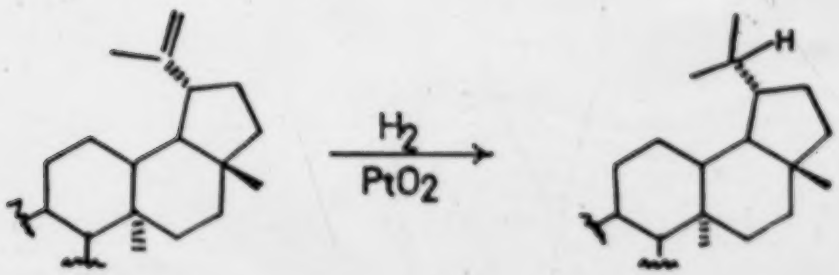


It is immediately obvious that a new fragmentation has been introduced, namely, loss of an isopropyl group. Furthermore the fragments which in O,O-diacetylcyathic acid occur at 203 and 218 are now in evidence at 205 and 220. The intensity of these fragments has also been reduced.

The P.M.R. spectrum shows a two hydrogen multiplet at δ 4.6. There is no vinyl methyl group at δ 1.67 and the aliphatic methyl envelope has been augmented by the addition of two methyls both appearing as doublets.

The I.R. spectrum of O,O-diacetoxydihydrocyathic is essentially identical to that of the parent acid. The carbonyl at 1685 cm^{-1} is still there.

Thus one can say that the following changes have occurred.



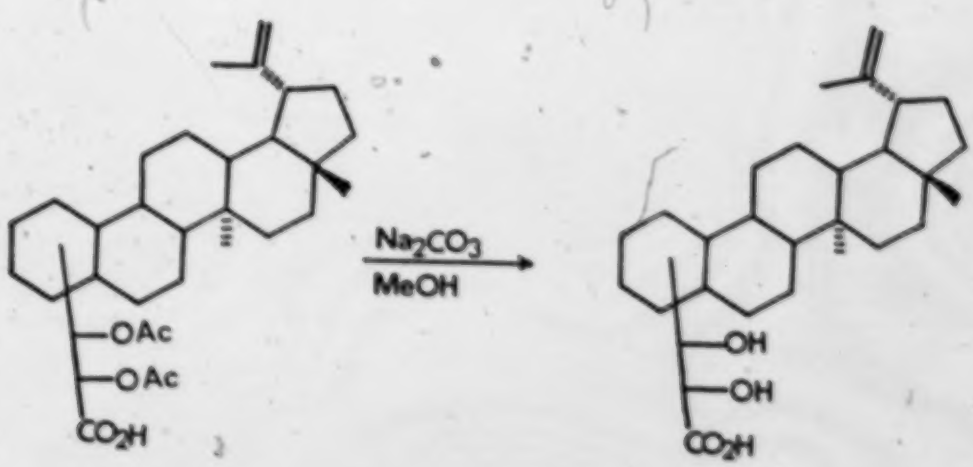
Treatment of O,O-diacetylcyathic acid with Na_2CO_3 in methanol for 48 hours yields a dihydroxycarboxylic acid. This acid we have named cyathic acid.

The I.R. spectrum of cyathic acid shows a strong hydroxy absorption at 3400 cm^{-1}. Also a single carbonyl occurs at 1685 cm^{-1}. The bands observed in the diacetyl derivative at 1740 cm^{-1} and 1250 cm^{-1} are no longer apparent in cyathic acid.

The P.M.R. spectrum of cyathic acid shows a broad doublet characteristic of a isopropenyl group at δ 4.6. This doublet integrates for two hydrogens. A complex multiplet corresponding to two hydrogens appears at δ 3.5.

The mass spectrum indicates a molecular formula of $C_{30}H_{48}O_4$.

Thus one can say that the following changes have occurred.

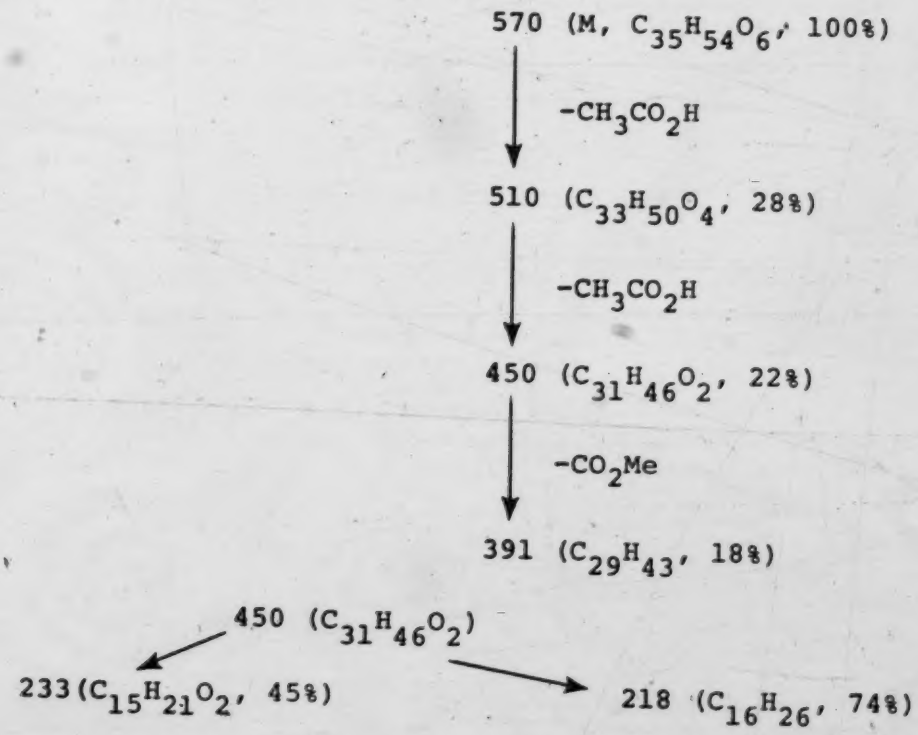


O,O-diacetylcyathic acid can be esterified using an excess of diazomethane in methylene chloride. In contrast to the normal speed of this reaction with ordinary carboxylic acids, in this case the reaction is slow and proceeds to completion over a 12 hour period. A quantitative yield of O,O-diacetylmethylcyathate is obtained.

The P.M.R. of this compound contains a methoxy methyl group at δ 3.7. The remaining part of the spectrum is identical to that of the parent acid.

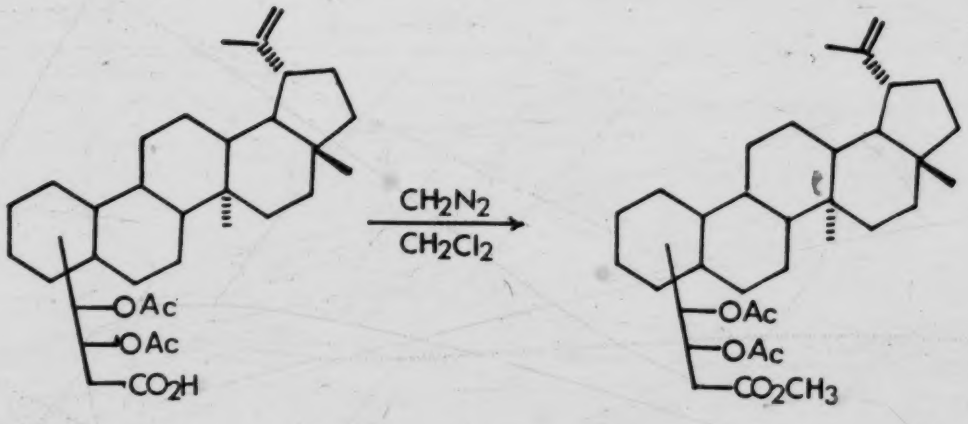
The I.R. spectrum shows a carbonyl at 1720 cm^{-1} in addition to the acetyl carbonyls at 1740 cm^{-1}. There is no apparent hydroxy absorption above 3000 cm^{-1}.

The mass spectrum offers clear proof that esterification has occurred. The molecular formula is $C_{35}H_{54}O_6$. The fragmentation pattern is as follows:



Thus the fragment at 218 representing the C, D and E rings is unchanged when compared to the free acid whereas the fragment at 233 represents an augmented 219 fragment. This clearly confirms the presence of all the oxygen functions in the A and B rings.

One can say, therefore, that the following changes have occurred.



Treatment of O,O-diacetylmethylcyathate with Na_2CO_3 in methanol for 48 hours gave methyl cyathate in good yield.

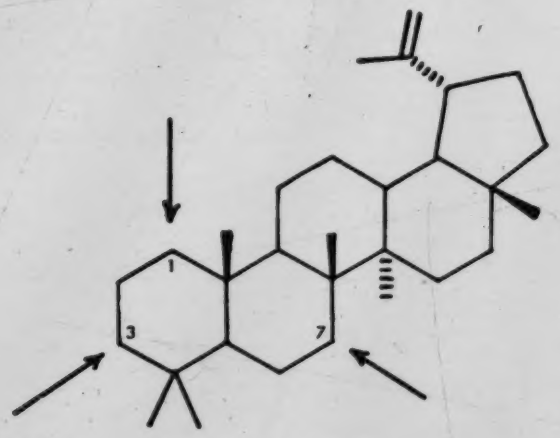
The I.R. spectrum of methyl cyathate shows a single carbonyl at 1715 cm^{-1} and a prominent hydroxy group at 3400 cm^{-1}. The band previously observed at 1250 cm^{-1}

due to the acetate groups is absent.

The P.M.R. spectrum shows an isopropylidene methylene at δ 4.6 as a doublet. A methoxy methyl appears at δ 3.7 as a sharp singlet. However between δ 3.2 and δ 3.6 there are two doublets of doublets. These correspond to hydroxy α-methine protons. They clearly indicate that both hydroxy groups are secondary. Furthermore, splittings of 11 Hz and 5 Hz for one and 12 Hz and 4.5 Hz for the other clearly demonstrates that these hydroxy groups are equatorial.

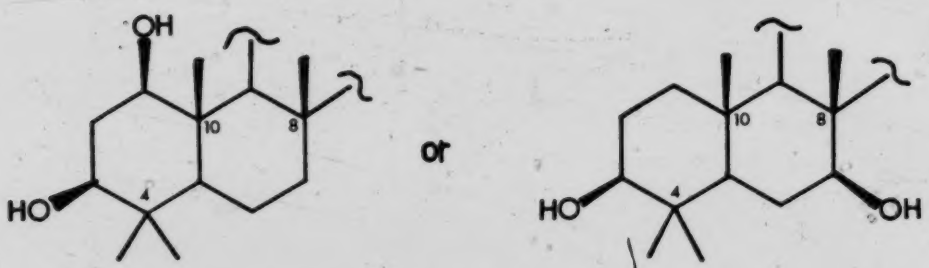
The mass spectrum corroborates a molecular formula of $C_{31}H_{50}O_4$. In other respects it is similar to that of cyathic acid.

One can now make some decision concerning the position of the hydroxy (and therefore also acetoxy) groups in the A and B rings. From the splittings observed in the P.M.R. of methyl cyathate these hydroxy groups can only be flanked by two vicinal hydrogens. In the lupane skeleton there are only three such positions.



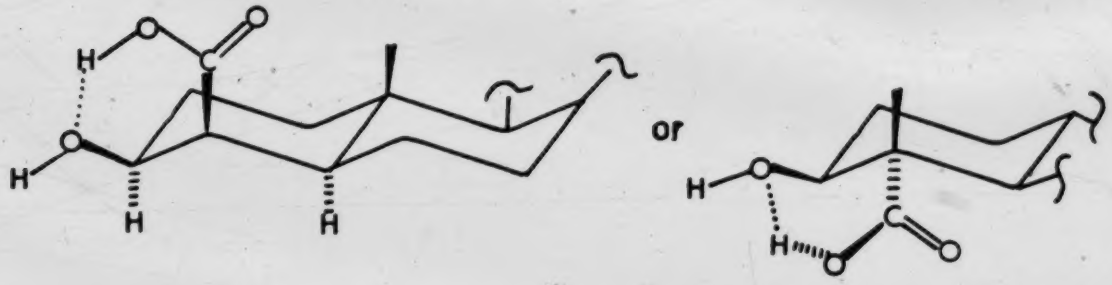
These are carbons-1, 3 and 7. The logical position for the hydroxy groups is carbons 1 and 3. This is in keeping with the observed oxygenation pattern found in Glochidonol (109).

The P.M.R. spectrum of methyl cyathate in pyridine-d_5 when compared to that run in $CDCl_3$ shows three distinct methyl shifts (0.4 ppm, 0.29 ppm and 0.25 ppm). To obtain three such shifts with only two hydroxy groups requires the presence of a hydroxy group at carbon-3. Furthermore, the magnitude of the shifts is in keeping with a (synclinal) gauche interaction between the hydroxy and methyl groups.[108] This interaction is apparent at all three possible sites. Thus we can write two partial structures

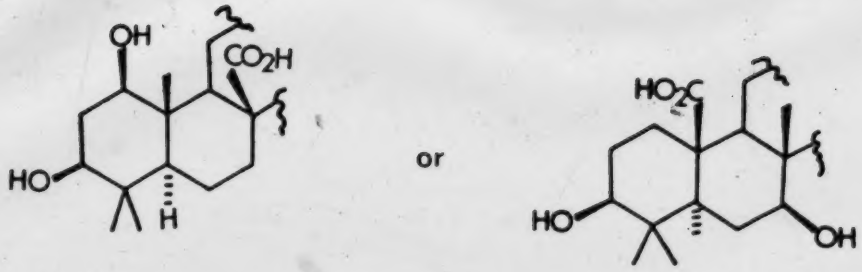


The carbon bearing the carboxyl group can be at carbon-8 or 10. This gives four possible structures. These can be whittled down as follows. Comparison of I.R.

spectrum of O,O-diacetylcyathic acid and cyathic acid shows no variation in the frequency of the carboxyl carbonyl. If the carboxyl were at either the α or β position on carbon-4 it should show a H-bonding effect on going from the acetate to the alcohol.

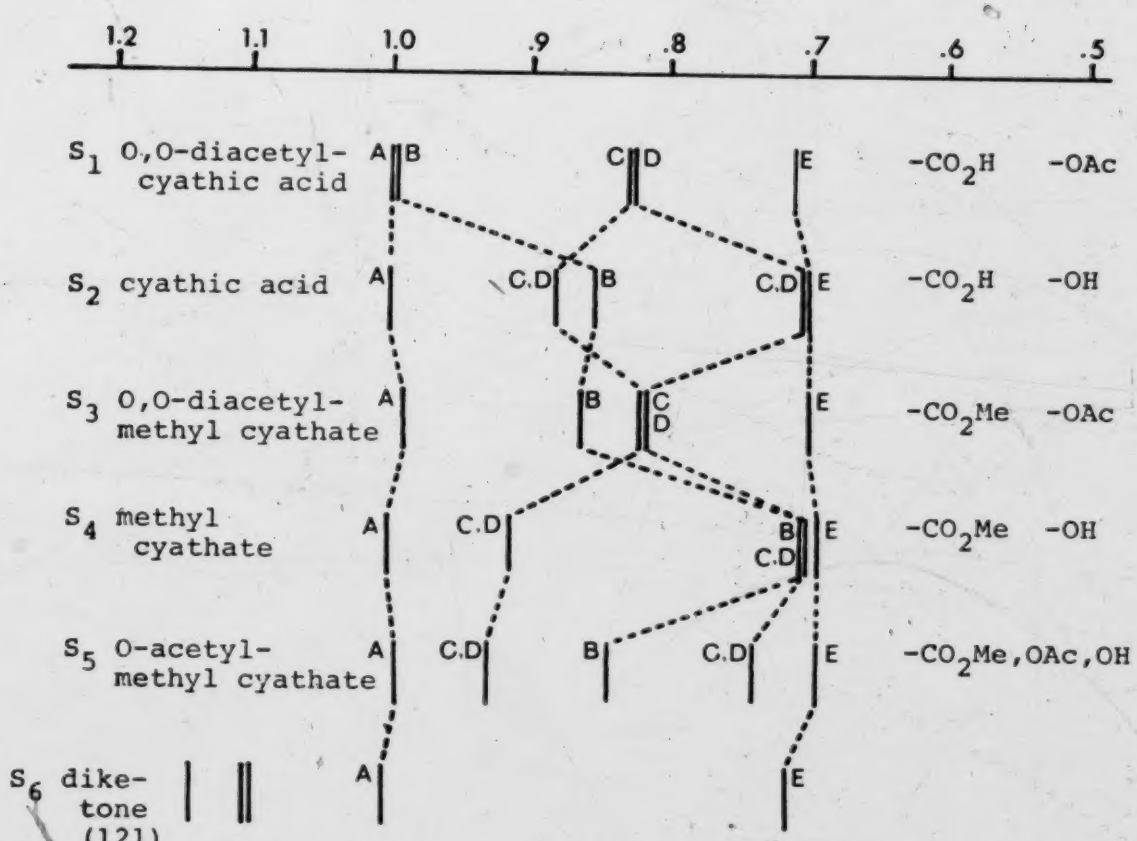


The lack of such an effect implies the absence of a carboxyl at C-4. This is in agreement with the earlier observation that a hydroxy group at C-3 interacts with two methyl groups. Furthermore it also becomes apparent that if the hydroxy group is at C-1 then the carboxyl must be at C-8 or if the hydroxyl is at C-7 then the carboxyl is at C-10. A hydroxy and carboxyl group adjacent to one another should show an obvious H-bonding effect. Thus there are only two possible structures for cyathic acid.

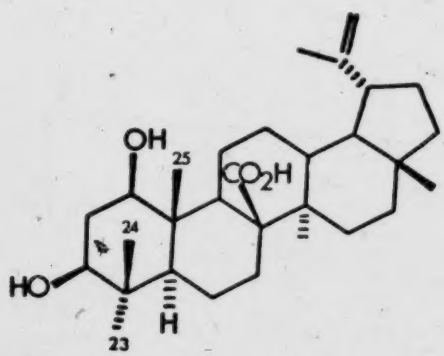


We can distinguish between these two possibilities as follows. A detailed examination of the aliphatic methyl group envelope of the derivatives prepared so far indicated a number of small but significant shifts in certain of the methyl groups.

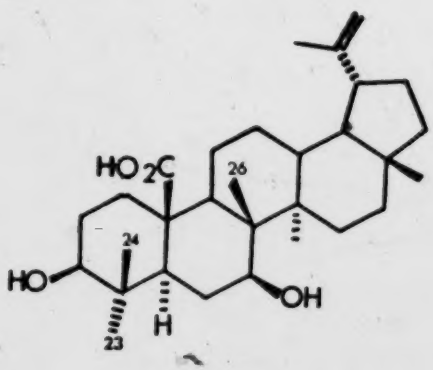
An expanded version of the methyl group envelope is shown in the next diagram. If one compares S_1 and S_3 it is obvious that methylation affects only one methyl group. This is confirmed by comparing S_2 and S_4 also. However, removing the acetyl groups affects three methyl groups, one of which is the same as that affected by methylation. This group is marked B in S_1 and S_2 and S_3 and S_4. Therefore one can say that removing one of the acetyl groups affects two methyls while the other affects only one. It becomes obvious that the acetoxy group that affects two methyl groups does not affect


1.2
1.1
1.0
.9
.8
.7
.6
.5
S1 O,O-diacetyl-cyathic acid
A B
C D
E
-CO2H -OAc
S2 cyathic acid
A
C.D B
C.D E
-CO2H -OH
S3 O,O-diacetyl-methyl cyathate
A
B
C D
E
-CO2Me -OAc
S4 methyl cyathate
A
C.D
B C.D
E
-CO2Me -OH
S5 O-acetyl-methyl cyathate
A
C.D
B
C.D
E
-CO2Me,OAc,OH
S6 dike-tone (121)
A
E

that methyl group which is moved by esterification when one compares S_2 and S_4. This is nicely confirmed by S_5. Here the methyl group marked B is unchanged compared to S_3 whereas the methyl groups marked CD are affected. Since the difference between S_3 and S_5 is removal of only one acetoxy group, this acetoxy group must affect the CD methyl groups and not the B methyl group. Thus one can choose between the two possible structures for cyathic acid as follows. In structure (119) esterification can only affect C-25, whereas in (120) it should



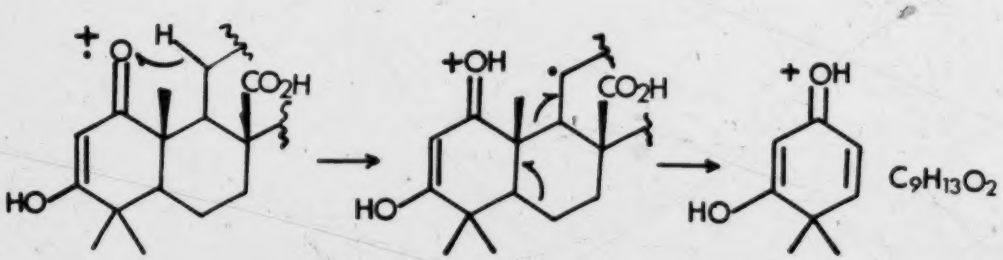
(119)



(120)

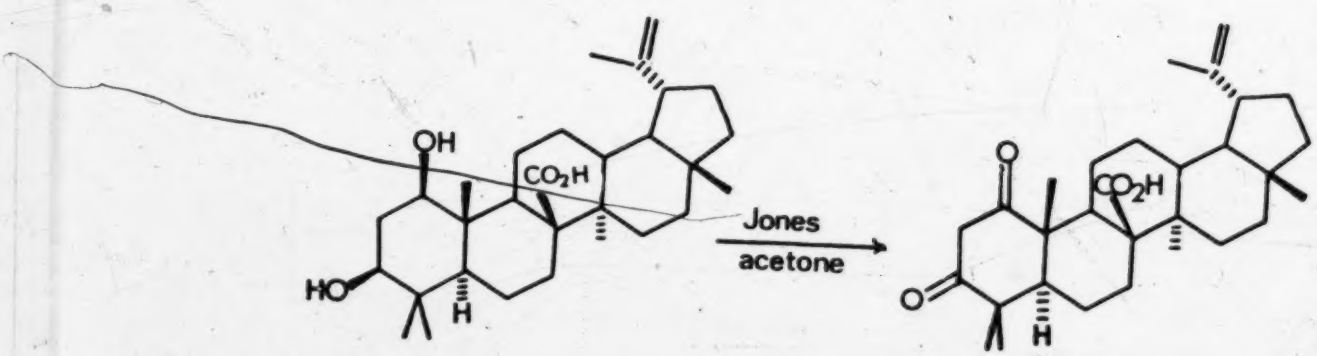
affect both C-24 and C-26. Furthermore in (120) one of the methyl groups affected by esterification is also part of a pair of methyl groups affected by acetylation of one of the hydroxy groups. This is in contradiction to the facts. Thus the P.M.R. evidence strongly supports structure (119).

A simple chemical experiment was used to add further weight to the evidence in favour of (119). Namely, oxidation of (119) should provide a 1,3-diketone whereas (120) should not. Oxidation of cyathic acid using Jones' reagent in acetone gave a single crystalline product (121). The I.R. spectrum contained carbonyls at 1720 cm^{-1}, 1698 cm^{-1} and 1676 cm^{-1}. No hydroxy group absorption was apparent. The mass spectrum confirmed a molecular formula of $C_{30}H_{44}O_4$, and also displayed a fragment at 153 with a formula $C_9H_{13}O_2$ and an abundance of 63%. This hitherto unobserved fragment must represent a reverse Diels-Alder cleavage across the AB ring junction, e.g.,



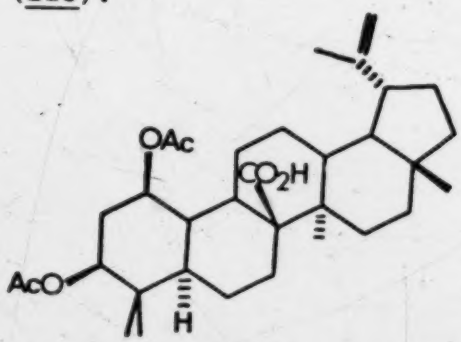
The P.M.R. showed a sharp singlet at δ 3.38, and the aliphatic methyl group envelope showed three strong downfield shifts when compared to cyathic acid. However it was the U.V. spectrum which clearly proved the structure of (121). This showed a band at 257 (ε = 10,000)

which shifted on the addition of base to 288 (ε = 26,000).

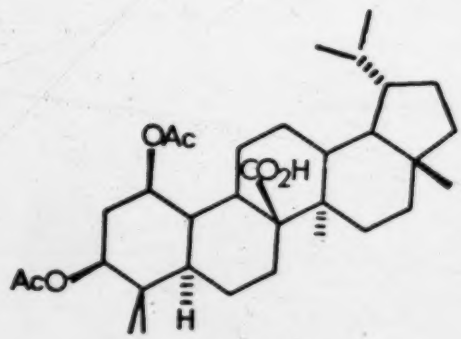


(119) (121)

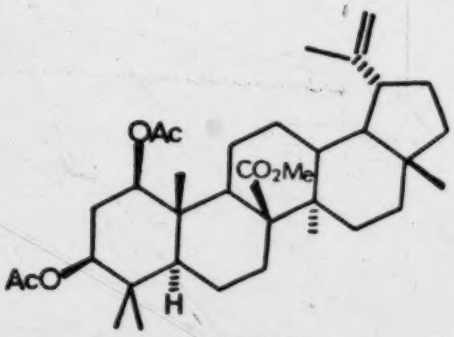
This key experiment proves the structure of cyathic acid to be (119). Furthermore the other compounds mentioned so far must be related as follows. O,O-diacetylcyathic acid is (122), O,O-diacetyldihydrocyathic acid is (123), O,O-diacetylmethylcyathate is (124) and methylcyathate is (125).



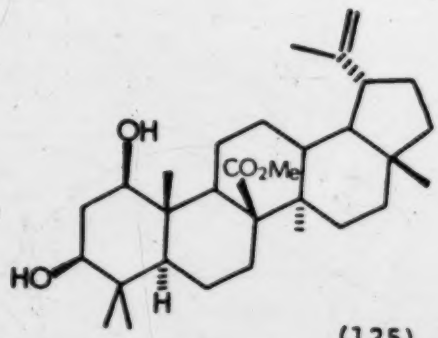
(122)



(123)

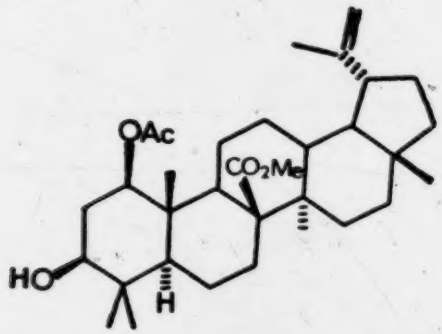


(124)



(125)

It was observed in the preparation of methyl cyathate (125) from O,O-diacetylmethylcyathate (124) that the reaction proceeded via an intermediate. It would seem reasonable that this intermediate should be the mono-acetate (126).



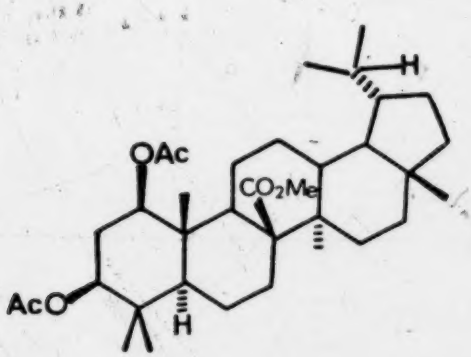
(126)

This is because the acetoxy group at C-1 is considerably more hindered than the one at C-3 in (125). Accordingly if the hydrolysis of (125) is carried out under shorter and milder reaction conditions, the product is exclusively the monoacetate (126).

The P.M.R. spectrum of (126) shows a one proton doublet of doublets at δ 4.6 corresponding to the C-1 methine proton. This proton shows coupling constants of 11 and 5 Hz confirming its axial nature. The C-3 methine proton resonates at δ 3.26, also as a doublet of doublets and with coupling constants of 12.5 and 4.5 Hz. A single acetoxy methyl group appears at δ 1.97.

The I.R. spectrum shows a hydroxyl absorption at 3500 cm^{-1} and carbonyl at 1718 cm^{-1}. The mass spectrum confirms the molecular formula of $C_{33}H_{52}O_5$.

Hydrogenation of (124) yielded O,O-diacetoxydihydromethylcyathate (127).



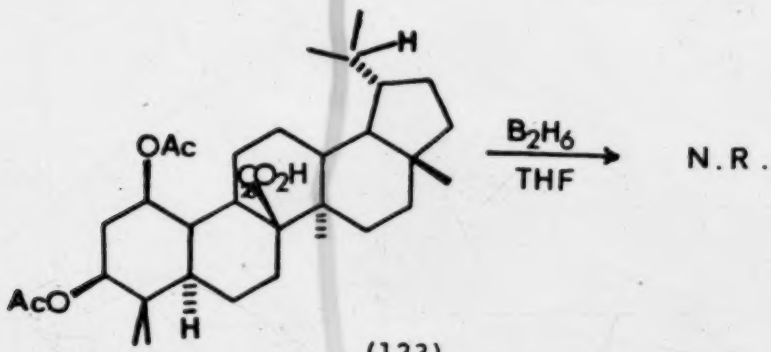
(127)

This molecule was made so that a clear picture of the C-1 and C-3 acetoxymethine protons could be obtained in the P.M.R. spectrum. In all 1,3-diacetoxy Δ20(29) derivatives made so far the isopropenyl hydrogens at C-29 obscured the 1,3-diacetoxy methine protons. However this problem is solved in (127) and the C-1 and C-3 methine protons appear as doublets of doublets at δ 4.56 and δ 4.63. The coupling constants are 10.8 and 5.7 at δ 4.63 and 12.2 and 5.3 at δ 4.56. This confirms the equatorial nature of both acetoxy groups.

The I.R. spectrum of (127) shows carbonyls at 1740 cm^{-1} and 1720 cm^{-1} and the mass spectrum confirms a molecular

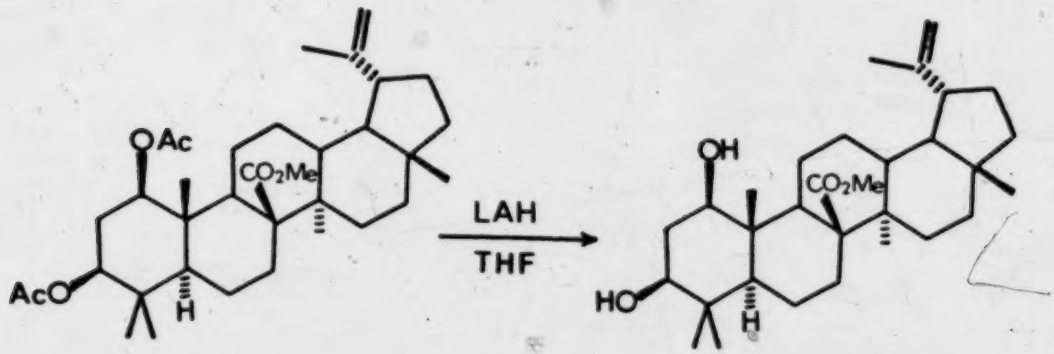
formula of $C_{35}H_{56}O_6$.

Attempts to change the functionality at C-26 were markedly unsuccessful. Thus treatment of O,O-diacetyl-dihydrocyathic acid (123) with diborane in T.H.F. gave only recovered starting material.



(123)

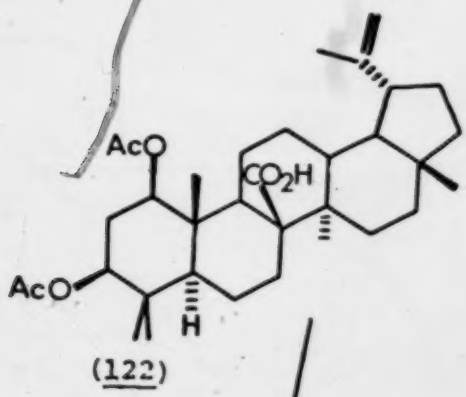
Furthermore treatment of O,O-diacetylmethylcyathate (124) with a large excess of lithium aluminium hydride in T.H.F. gave methyl cyathate (125) as the sole product in 92% yield.



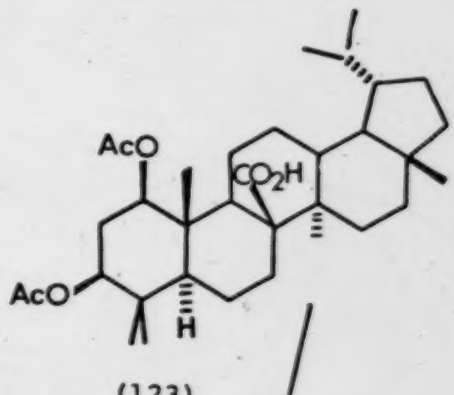
(124) (125)

This gives a clear indication of the highly hindered nature of the carbomethoxy group at C-8. In retrospect some interesting aspects of the physical properties of cyathic acid derivatives can now be rationalized with these structures.

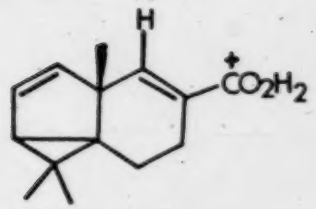
In the mass spectra of O,O-diacetylcyathic acid (122) and O,O-diacetyldihydrocyathic acid (123) there is a marked difference in the relative populations of those fragments due to C, D and E ring as compared to the A and B. Thus in (122) the fragments at 218 and 203 are more prevalent than the 219 fragment. In (123) it is the other way round.



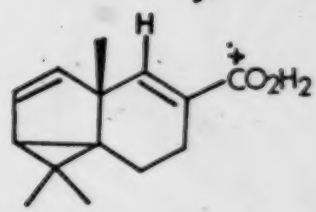
(122)



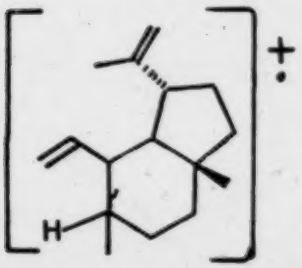
(123)



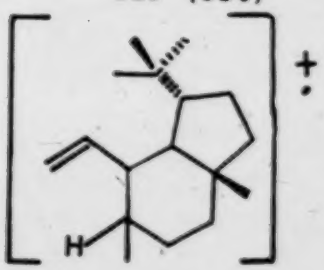
219 (34%)



219 (33%)

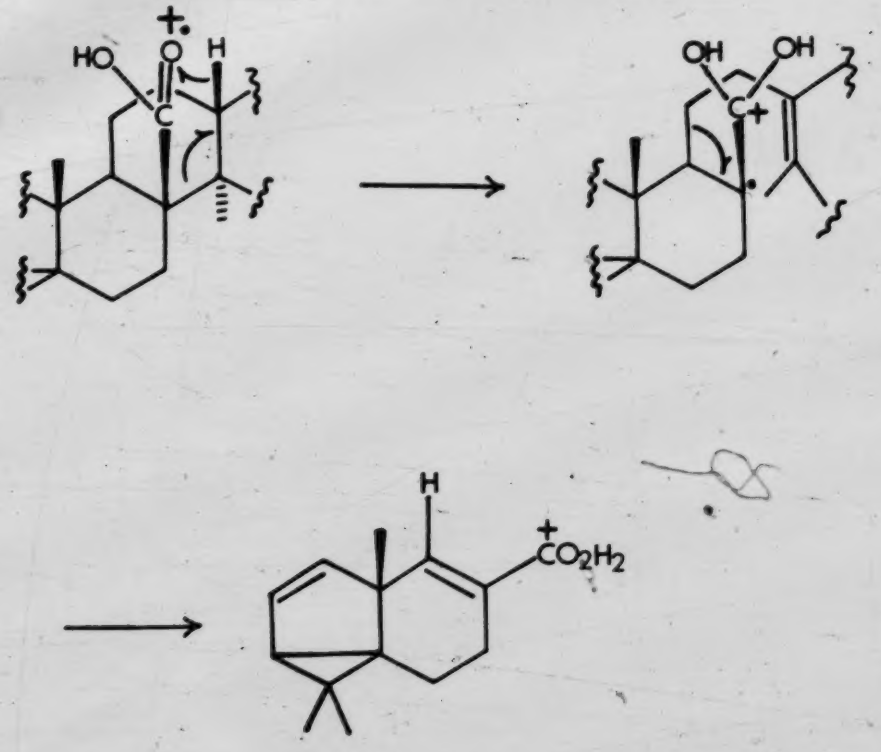


218 (100%)



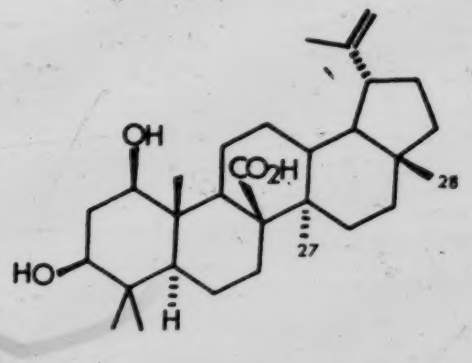
220 (10%)

This is in keeping with the observation[109] that in the fission of triterpenes in the mass spectrometer that portion of the molecule with the least electronegative substituents generally carries the charge. Thus in (122) 218 predominates over 219. However in (123) the ability of the C, D, E ring fragment to carry any charge at all is diminished by removing the double bond and the A, B ring fragment predominates. The genesis of the A, B ring fragment at 219 is explained as follows.



(219)

Some comments can be made concerning the P.M.R. spectra of cyathic acid derivatives. It has already been stated that in the aliphatic methyl group envelope two methyl groups remained constant while three others were observed to undergo various shifts in these derivatives. These two methyl groups (at δ 1.0 and δ 0.7) must therefore represent C-27 and C-28 of cyathic acid.



(119)

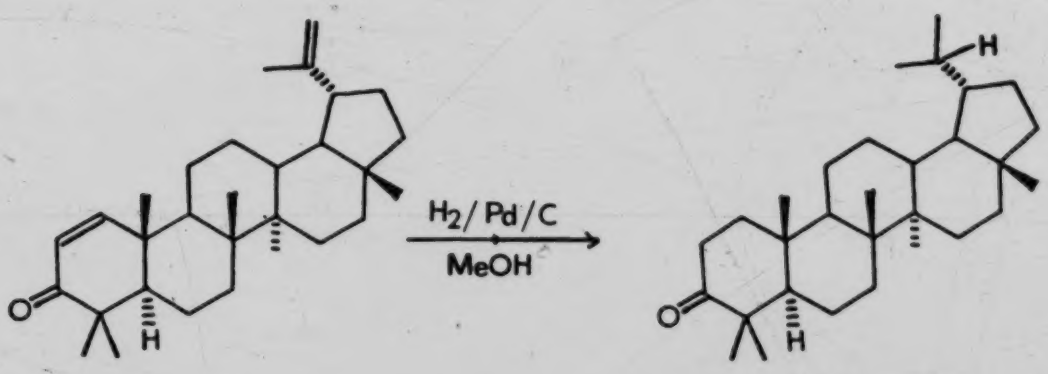
In a comprehensive study of lupane triterpenes[110] the average value observed for C-28 and C-27 was δ 0.75 and δ 0.95 respectively. This is in excellent agreement with the values observed for cyathic acid.

In the infrared spectra of cyathic acid the value observed for a carboxy group or the ester group at C-8 is 1685 cm^{-1} and 1718 cm^{-1} respectively. These values are approximately 20 cm^{-1} lower than the normal values for these groups. This is in keeping with the highly hindered nature of a carboxyl at C-26. A similar

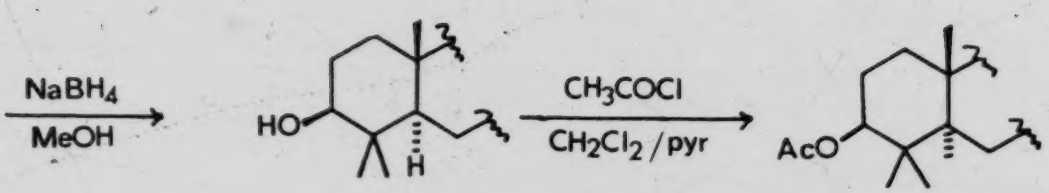
observation can be made about the acetoxy groups at C-1 and C-3. Thus an acetoxy at C-3 is normal and absorbs at 1740 cm^{-1} whereas an acetoxy at C-1 is hindered and absorbs at 1720 cm^{-1}.

In order to correlate the cyathic acid skeleton with the lupane skeleton it was decided to measure the carbon-13 magnetic resonance spectra of a number of cyathic acid derivatives and also some lupane derivatives. The lupane derivatives were synthesized from the fungal metabolites glochidone (108) and glochidonol (109). The latter two compounds were isolated along with cyathic acid.

Thus glochidone (108) was hydrogenated using palladized charcoal in methanol to give lupanone (128). Lupanone (124) was reduced to lupanol (129), which was then acetylated to give lupanol acetate (130).

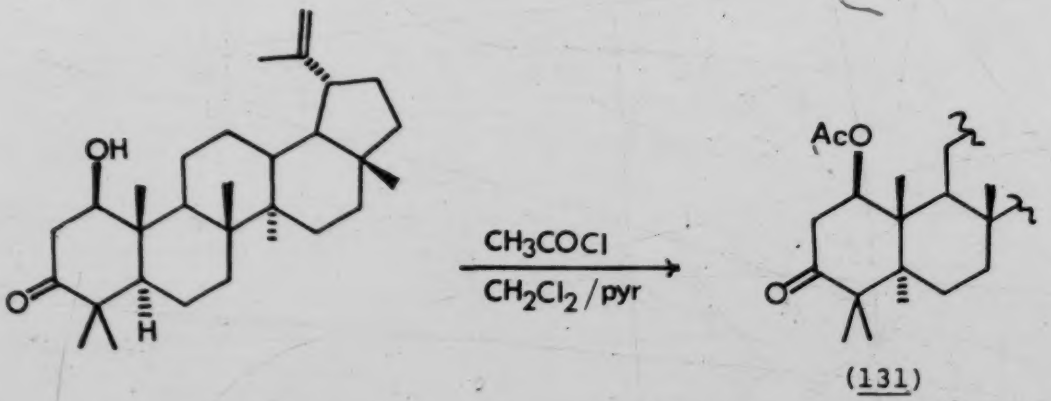


(108) (128) 90%

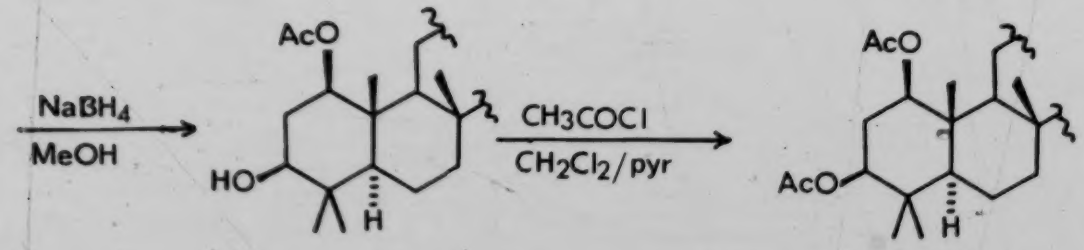


95% (129) (130) 90%

Glochidonal (109) was acetylated using acetyl chloride/pyridine to give glochidonyl acetate (131), which was then reduced using $NaBH_4$ to give 1,β-acetoxy-3,β-hydroxylupene (132). This was then acetylated to give 1,β-3,β-diacetoxylupene (133).

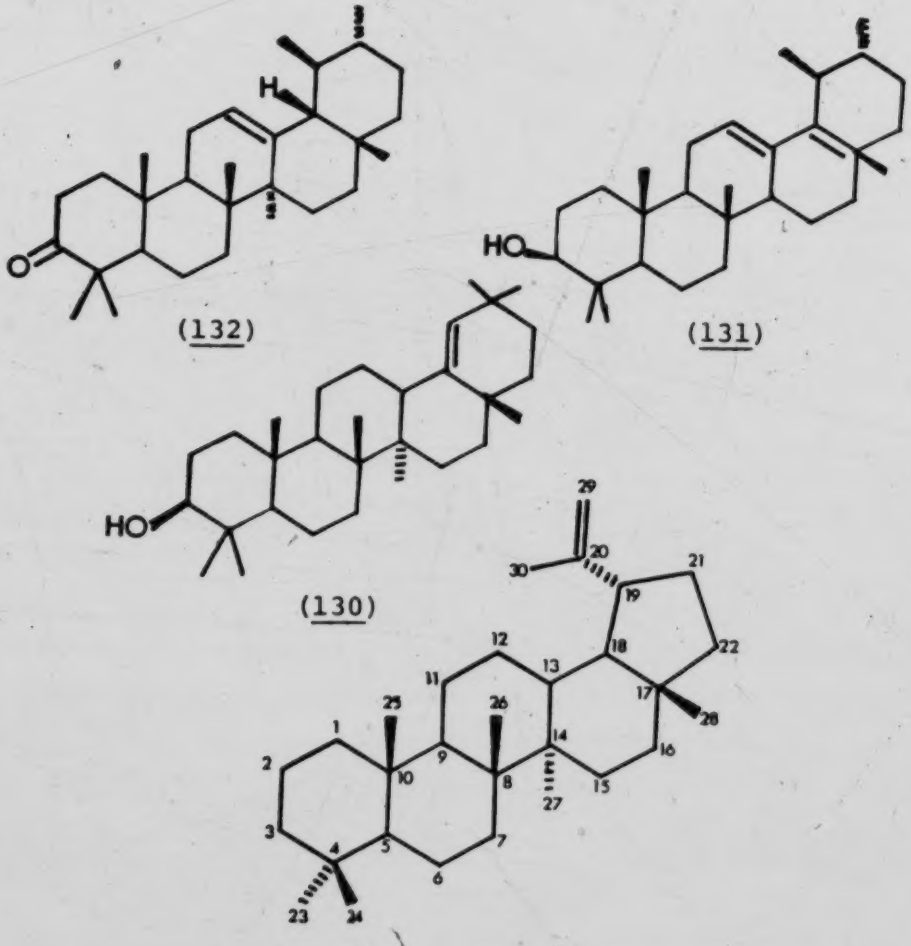


(131)



(132) (133)

Furthermore, the literature contained three pentacylic triterpenes whose C.M.R. spectra had been assigned. These were germanicol (130),[111] β-amyrin (131)[112] and the corresponding ketone (132).



(132) (131) (130)

The various carbons in the lupane skeleton were assigned in the following manner. Carbons 1 to 6 and 9 to 11 were easily assigned on the basis of the shifts

induced by the various chemical transformations of the A ring. Thus the presence of a ketone at the 3-position causes a well documented effect at carbons 4, 5 and 6. Similarly a substituent at C-1 causes a profound effect at carbons 9, 10 and 11. Carbons 7 and 8 were assigned using the observed literature values[113] coupled with their relative inertness to changes in the A ring. Carbon 12 was easily recognized by the strong upfield shift induced there by hydrogenation of the isopropylidene double bond. Carbons 13, 14, 15 and 16 were assigned by comparison with the known literature values for these positions in conjunction with their relative permanence. Carbon 17 is the only remaining unassigned singlet. Carbons 18 and 19 are both doublets but carbon 19 is affected by hydrogenation of isopropylidene double bond. In a similar fashion carbons 21 and 22 are the only remaining triplets but carbon 21 is slightly shifted on hydrogenation of the double bond. The three A ring methyl groups are easily determined from their characteristic shifts on changing the substituents in that ring. Carbons 27 and 28 are assigned because they display the largest and smallest couplings respectively in the off-resonance mode. This is based on the fact that the coupling observed in the off-resonance mode is directly proportional to the frequency difference between the decoupler frequency and the position of that carbon's

hydrogens in the P.M.R. spectrum. Since carbon 28 is the highest field methyl group and carbon 27 the lowest in the P.M.R. (as already discussed) they respresent the extremes in off-resonance splitting patterns. The only remaining methyl group C-26 is thus also assigned.

Thus the following values were determined for the aforementioned lupane derivatives.

	108	128	129	130	109	131	132	133
1	159.6	39.6	38.8	38.4	79.6	80.4	80.9	80.3
2	125.1	33.6	27.4	23.7	45.2	41.8	33.4	29.9
3	205.2	217.7	79.0	81.0	216.1	215.2	75.0	78.4
4	44.6	47.3	38.9	37.8	47.1	46.8	38.7	37.8
5	53.4	54.9	55.3	55.4	51.4	50.8[E]	53.2	53.0
6	19.2	19.7	18.4	18.2	19.6	19.5	17.8	17.7
7	33.7	34.1	34.4	34.3	33.0	32.7	34.0	34.0
8	41.7	40.8	40.9	40.9	41.2	41.0	41.3	41.3
9	44.4	44.7	44.7	44.7	50.8	50.3[E]	51.0	51.0
10	39.5	36.8	37.2	37.1	43.0[D]	41.9	42.8	42.2
11	21.2	21.5	21.0	21.0	23.0	22.4	22.9	22.8
12	25.0	21.9	21.9	21.9	25.2	25.2	25.0	25.0
13	38.2	37.9	37.8	37.8	38.0	38.0	37.4	37.4
14	42.7[A]	43.1[A]	43.2[A]	43.2	43.0[D]	42.9[A]	42.9[A]	42.9[A]
15	27.3	27.3	27.4	27.4	27.5	27.5	27.5	27.5
16	35.4	35.5	35.6	35.6	35.6	35.5	35.6	35.6
17	43.0[A]	43.1[A]	43.0[A]	43.0	43.0[D]	42.9[A]	42.9[A]	42.9[A]

continued.....

	108	128	129	130	109	131	132	133
18	48.1[B]	47.6	47.6	47.6	48.3[B]	48.1[B]	48.2[B]	48.2[B]
19	47.8[B]	49.4	50.2	50.0	48.0[B]	48.0[B]	48.0[B]	48.0[B]
20	150.5	29.4	29.4	29.6	150.7	150.5	150.6	150.6
21	29.8	26.8	26.8	26.8	29.8	29.8	29.9	29.9
22	39.9	39.6	40.4	40.4	40.0	39.9	39.8	40.0
23	27.8	26.7	28.0	28.0	28.0	28.6	27.8	27.8
24	21.4	21.0	15.4	16.6	19.9	19.7	14.9	16.0
25	19.0	15.2	15.2	15.2	11.86	12.8	13.2	13.2
26	14.4	14.4	14.5	14.4	14.5	14.4	14.4	14.4
27	16.4	15.9	16.0	16.0	16.0	15.8	16.2	16.1
28	18.0	18.1	18.1	18.1	18.1	18.0	18.0	18.0
29	109.5	23.0[C]	23.0[C]	23.0[C]	109.5	109.6	109.5	109.5
30	19.3	15.8[C]	16.1[C]	16.1[C]	19.3	19.3	19.2	19.2
C_1-$C\underline{H_3}CO$						21.6	21.9	21.8
C_1-$CH_3\underline{CO}$						170.3	170.5	170.2
C_3-$C\underline{H_3}CO$				21.3				21.1
C_3-$CH_3\underline{CO}$				170.9				

A - carbons 14 and 17 may be interchanged

B - carbons 18 and 19 may be interchanged

C - carbons 29 and 30 may be interchanged

D - carbons 10 and 14 may be interchanged

E - carbons 5 and 9 may be interchanged

As a check on these assignments an empirical calculation was undertaken using a new method developed by

Beierbeck[111] for carbon thirteen resonances. This method does not allow calculations to be made for all the carbons of the lupane skeleton. For those to which it applies however it lends support to the assignments. As can be seen below correlation is very good indeed.

C^{13} Spectrum of Glochindonol (109)

Carbon	calc.	obsd.
1	81.04	79.6
2	41.14	45.15
3	212.76	216.11
4	48.63	47.15
5	55.50	51.4
6	17.62	19.64
7	33.12	33.02
8	41.53	41.16
9	48.49	50.7
10	42.16	42.99
11	25.03	23.03
12	26.72	25.19
13	43.35	38.03
14	41.33	42.99
15	28.57	27.51
16	37.67	35.5
18	47.92	48.30

The C.M.R. spectra of four cyathic acid derivatives were measured. The assignments were made following the

same principles used for the lupane series of derivatives. In general no difficulty was encountered as the vast majority of resonances were identical in value to those observed in a similar lupane derivative. In this fashion the following values were observed for four cyathic acid derivatives.

	(122)	(124)	(126)	(125)
1	78.1[A]	78.0[A]	79.0	75.8[A]
2	30.0	30.0	32.8	37.0
3	76.3[A]	76.2[A]	74.4	74.7[A]
4	37.7	37.7	38.6	38.3
5	53.8[C]	53.8[C]	53.8[C]	53.6
6	19.6[D]	19.6[D]	19.3[D]	19.4[D]
7	30.2	30.4	30.3	30.5
8	53.9[C]	53.8[C]	53.8[C]	53.6
9	52.4	52.4	52.5	53.0
10	42.7[B]	42.5[B]	42.5[B]	43.8
11	23.4	23.6	23.6	24.2
12	24.3	24.3	24.2	24.2
13	36.5	36.7	36.7	36.9
14	42.9[B]	42.9[B]	42.8[B]	42.5[E]
15	30.7	30.7	30.6	30.5
16	35.2	35.2	35.0	34.9
17	42.9[B]	42.7[B]	42.7[B]	42.1[E]
18	48.4	48.4	48.2	48.5
19	47.8	47.8	47.6	47.9
20	150.4	150.4	150.5	150.1
21	29.8	30.0	29.8	29.5

continued.....

	(122)	(124)	(126)	(125)
22	39.6	39.6	39.4	39.2
23	27.8	27.8	27.5	27.2
24	15.9	15.9	14.6	14.2
25	11.9	11.9	11.6	10.0
26	170.5	175.9	176.3	175.8
27	16.1	16.0	15.7	15.2
28	17.7	17.8	17.6	17.0
29	109.4	109.6	109.3	108.8
30	19.3[D]	19.3[D]	19.3[D]	19.2[D]
C_1-$\underline{CH_3}CO$	21.7	21.7	21.6	
C_1-$CH_3\underline{CO}$	170.5	170.2	171.2	
C_3-$\underline{CH_3}CO$	21.0	21.0	-	
C_3-$CH_3\underline{CO}$	181.3	181.4	-	
CO_2-CH_3	50.9	50.9	50.7	49.9

A - carbons 1 and 3 may be interchanged

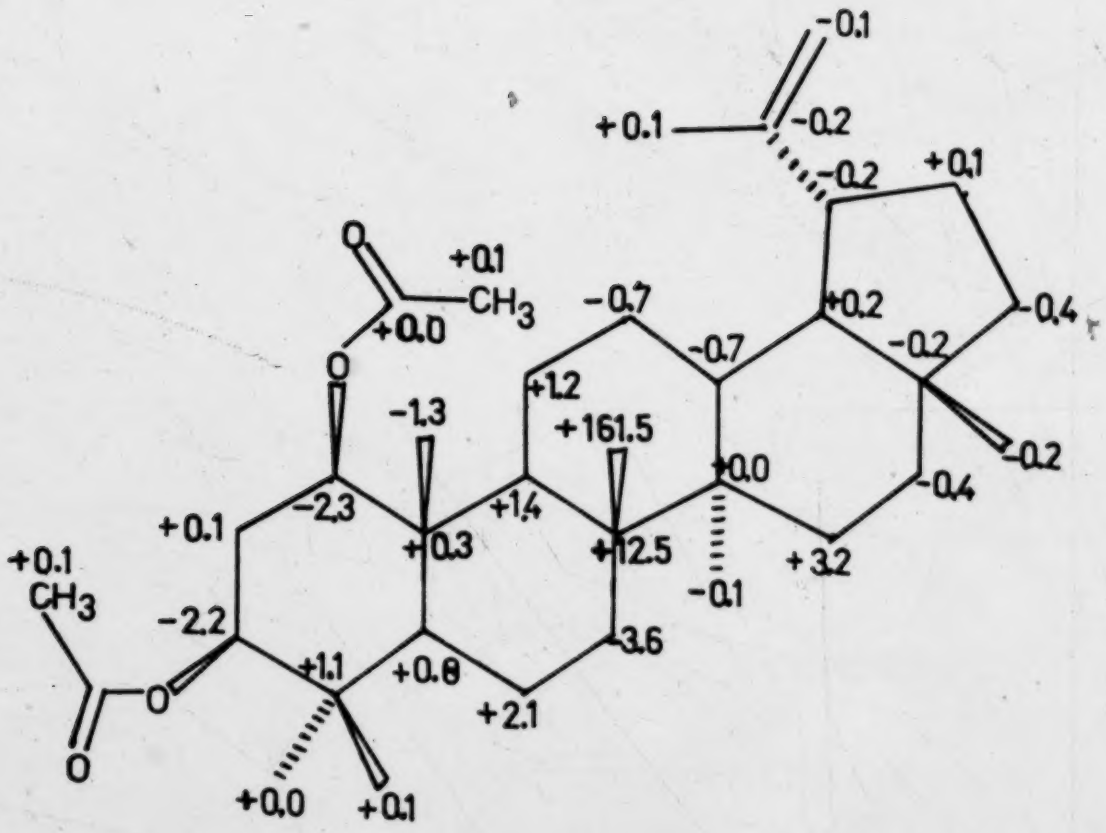
B - carbons 10, 14 and 17 may be interchanged

C - carbons 5 and 8 may be interchanged

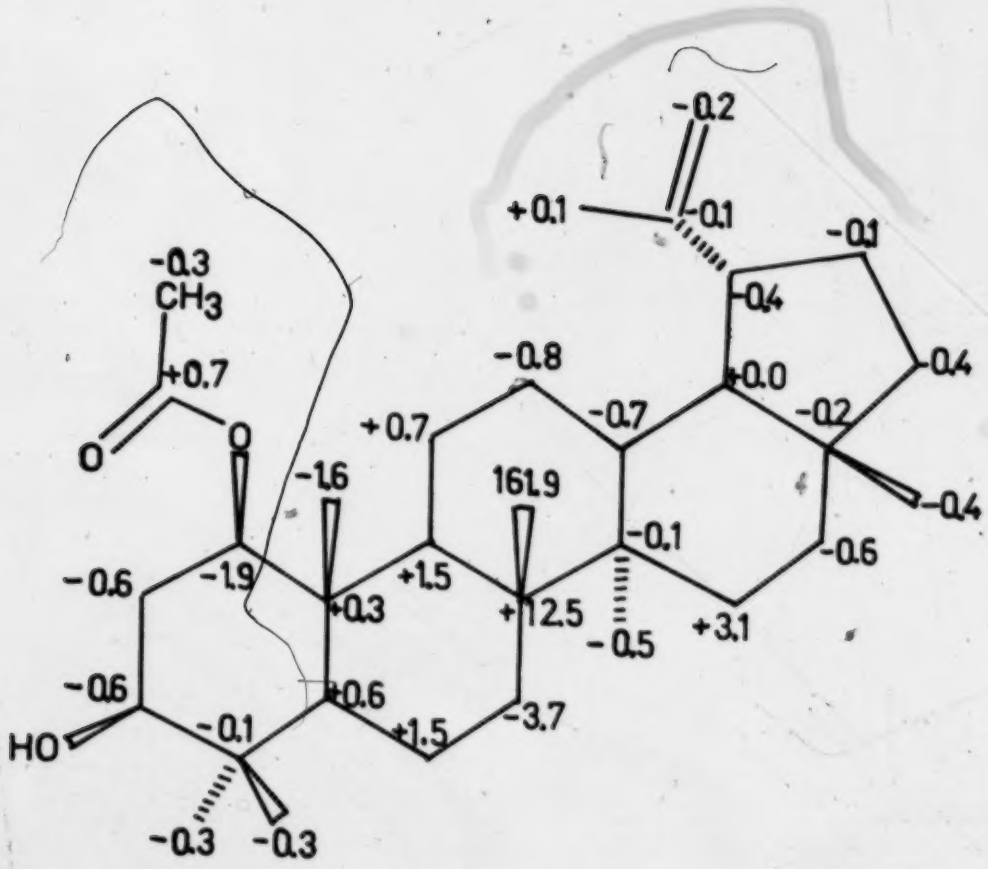
D - carbons 6 and 30 may be interchanged

E - carbons 14 and 17 may be interchanged

It now becomes of interest to compare the two series of derivatives. If one compares O,O-diacetylmethylcyathate (124) and 1,β-3,β-diacetoxy lupene (133), one can say that the introduction of a carbomethoxy group causes the following perturbations of the lupene skeleton



If one compares 1,O-acetoxymethylcyathate (126) and 1,β-acetoxy-3,β-hydroxy-lupene (132) one can observe the same perturbations of the lupene skeleton

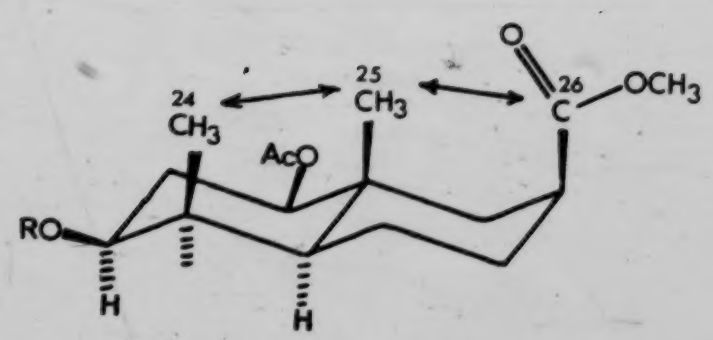


If one discounts any perturbations of less than 1 ppm then the following consistent shifts are observed.

	(124) → (133)	(126) → (132)
C-1	-1.9	-1.9
C-3	-2.2	-
C-6	+2.1	+1.5
C-7	-3.6	-3.7
C-8	+12.5	+12.5
C-9	+1.4	+1.5
C-15	+3.1	+3.1
C-25	-1.6	-1.6
C-26	+161.5	+161.9

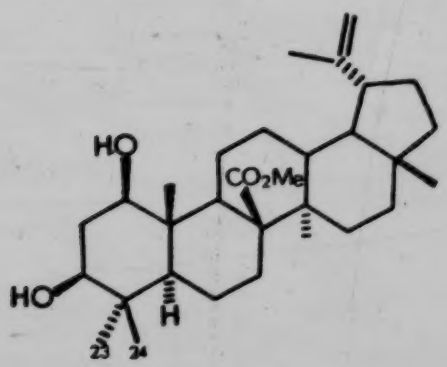
It can be seen immediately that exactly similar perturbations are observed in both series of compounds. This is despite the fact that sizable shifts are occuring in both series. However, because these shifts occur to an equal extent in both series the observed perturbation remains the same. This is excellent corroboration for the lupane skeleton of cyathic acid.

The perturbations themselves are also in keeping with a lupane skeleton for cyathic acid. They can be divided into two groups. The first group consists of perturbations at C-1 and C-3. It is very possible that these are due to slight deformations of the A ring transmitted via the 1.3 diaxial interactions of C-26 and C-25 and also C-25 and C-24.

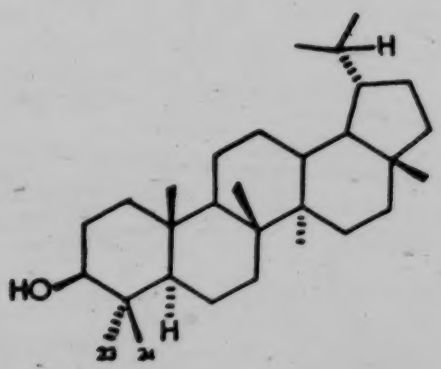


It has been shown[115] that the A ring of pentacyclic triterpenes is indeed sensitive to such interactions. However it is the changes about C-8 that are the most important. C-8 itself shows a downfield shift of 12.5 ppm. The observed average value for the shift at the β-carbon on changing a methyl group to a carbomethoxy is +12.0 ppm.[116] The effects observed at C-7 and C-9 are probably inductive effects with an added stereochemical effect at C-7 due to the loss of 1,3 diaxial H-interaction.[111] The effect at C-15 is surely a γ-gauche interaction. The effect at C-25 is a classic example of an upfield shift caused by steric compression. The preparation of these lupane derivatives also permitted us to make some correlation between the P.M.R. spectra of lupanes and cyathic acid derivatives.

Thus methylcyathate (125) shows three downfield shifts of methyl groups if one compares the P.M.R. spectrum run in $CDCl_3$ and pyridine-d_5. Lupanol (129) shows two such shifts of similar magnitude.



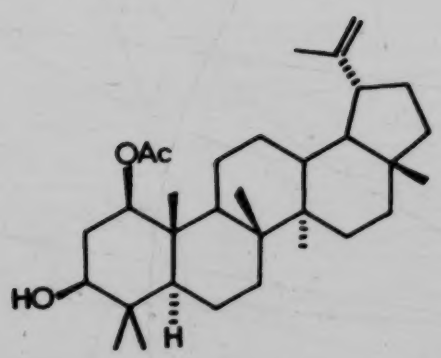
(125)

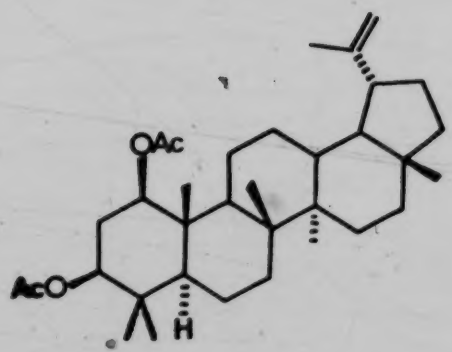


(129)

(125)	(125)	(129)
	+0.32	+0.32
	+0.38	+0.37
	+0.25	

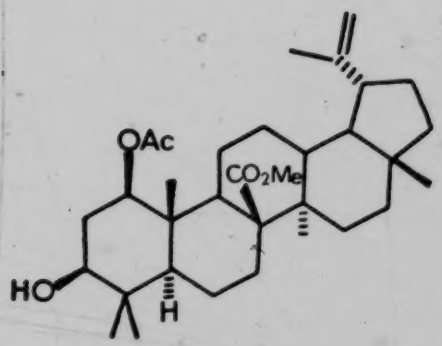
If one compares the P.M.R. of (132) and (133) one observes the following shifts

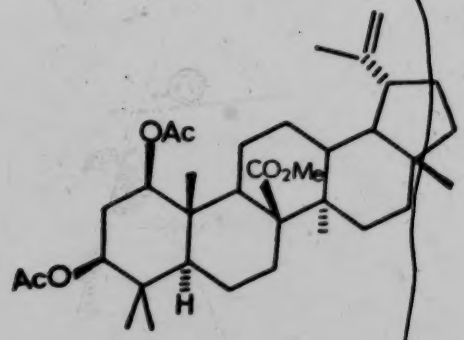




	(132)	(133)	
C_{25}	δ1.03	δ1.04	+0.01
C_{27}	δ1.00	δ1.03	+0.03
C_{26}	δ0.96	δ0.93	-0.03
$C_{24/23}$	δ0.92	δ0.84 (2x)	-0.08,+0.06
C_{28},$C_{23/24}$	δ0.78 (2x)	δ0.78	+0.00

A similar effect is observed in the cyathic acid series.

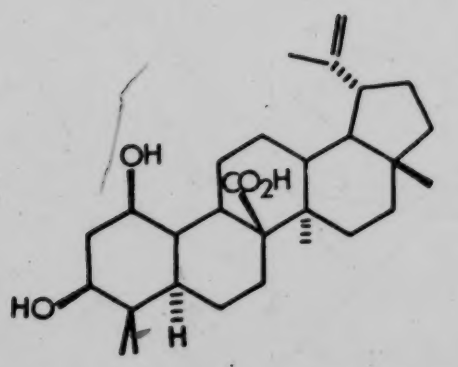




	(126)	(125)	
C_{27}	δ0.98	δ0.98	+0.00
$C_{24/23}$	δ0.94	δ0.85	+0.02
C_{25}	δ0.83	δ0.82 (2x)	-0.12,+0.07
$C_{23/24}$	δ0.75		
C_{28}	δ0.70	δ0.70	+0.00

In both series acetylation of the C-3 hydroxy group causes an upfield and a downfield shift in the C-23, 24 pair of methyl groups. Thus in the C-3 acetate both C-23 and C-24 become coincident.

This is yet another piece of evidence to prove that cyathic acid (119) has the structure shown.



(119)

EXPERIMENTAL

Melting points were determined on a Fisher Johns hot stage melting apparatus and are uncorrected.

Infrared spectra were recorded on a Nicolet 7199 FT-IR.

Proton magnetic resonance spectra were measured using a Varian Associates model HR-100 spectrometer interfaced in the Fourier mode to a Digilab FTS/NMR 3 data system.

Carbon magnetic resonance spectra were measured using a Brucker HFX-90 spectrometer or a Bruker WP-60 spectrometer. In either case the Fourier mode was used.

Mass spectra were recorded on an A.E.I. MS-50 mass spectrometer.

O.R.D. spectra were measured on a Jasco O.R.D./C.D. 55-20-2 (modified) machine.

U.V. spectra were determined on a Cary Model 15 U.V. and visible spectrometer.

Microanalyses were performed by the microanalytical laboratory of this department.

Thin layer chromatography was done generally on micro plates (75 x 25 mms) using Merck Silica Gel G (Type 60) and using General Electric Type 118-2-7 Electronic Phosphor as a fluorescent indicator. Plates were examined with U.V. light at a wavelength of 254 n.m.

Isolation of O,O-diacetylcyathic acid (122)

A flask containing 150 mls of Y.M. medium (4 gms yeast extract, 4 gms glucose, 10 gms malt extract per litre) was autoclaved at 121°C for 20 minutes. It was then inoculated with *Cyathus striatus* (strain No. 68037-II) and maintained at room temperature on a rotary shaker for five days. Subsequently, sterile pyrex glass beads (approximately 20) were added and the shaking continued for a further four days. Meanwhile four more flasks each containing 150 mls of sterile Y.M. medium and glass beads were prepared. When the first growth (known as G-I) had reached maturity it was used to inoculate the four secohdary flasks by adding 10 mls of culture medium from G-I to each. The flasks (now known as G-2) were maintained at room temperature on the rotary shaker for eight days. When mature the entire G-2 contents were added to 10 litres of Y.M. medium in a New Brunswick microferm apparatus. Two mls of polyol antifoam were added initially and the mycelia grown at 22°C with mechanical stirring (200 r.p.m.) and an aeration rate of 3 litres air/minute.

The myclia were harvested after 8 days and collected via centrifugation at 1000 G. using a high speed cream separator. The cells were dried between absorbent paper and then placed in a 1 litre Erlenmeyer flask which was

then filled with methanol. The methanol was boiled for 20 minutes and then decanted off. This procedure was repeated twice more. The combined methanolic extracts were concentrated under reduced pressure to yield 21 gms of crude material. Examination of this extract by T.L.C. (silica gel/chloroform/methanol) indicated that the majority of it was extremely polar material, e.g., sugars. The extract was triturated with 40 mls of methanol and concentration of this methanol solution *in vacuo* gave 4.5 gms of material.

This material was applied to a column (75 cms x 2.5 cms) of Sephadex LH-20 as a methanol solution. The column was then eluted with methanol at the rate of 20 mls/hour. About one hundred fractions of 5 ml each were collected. These fractions were allowed to concentrate by evaporation for two days. By this time it became apparent that the eluted material was concentrated in three sets of fractions. Fractions 51 to 55 jelled on standing. This behaviour is characteristic of glochidonol (109). Combination of these fractions and concentration yielded 200 mgms of crude glochidonol (109). This glochidonol could not be crystallized but was identified by its spectral properties[114] and by comparison with an authentic sample.

Combining fractions 38 to 41 yielded Glochidone (108) (40 mgms). This was identified by comparison with

an authentic sample.

Fractions 43 to 49 were combined and concentrated to yield crude O,O-diacetylcyathic acid (122). This was crystallized from 5 mls of methanol at 4°C to yield white amorphous crystals (90 mgms). Concentration of the mother liquors yielded a further quantity (60 mgms). Recrystallization of both quantities from methanol at room temperature over 8 days yielded pure O,O-diacetyl-cyathic acid (150 mgms) as clear white needles. These needles sublimed at 280°C and melted at 311-12°C.

Anal: calc. C, 72.35 H, 9.41
found C, 72.39 H, 9.46

P.M.R. ($CDCl_3$): δ 4.8-4.4 (m, 4H, C<u>H</u>-OAc, $R_2C{=}C\underline{H}_2$) δ 2.0 (s, 3H, $C\underline{H}_3{-}CO$), δ 1.97 (s, 3H, $C\underline{H}_3{-}CO$), δ 1.67 (b.s., 3H, $C\underline{H}_3{-}C{=}C$), δ 1.0 (s, 6H, $CH_3{-}CR_3$), δ 0.82 (s, 6H, $CH_3{-}CR_3$), δ 0.71 (s, 3H, $CH_3{-}CR_3$).

I.R. (film cast): 3500-3100 cm^{-1} (b, OH); 3080 cm^{-1} (w, $C{=}CH_2$); 2900 cm^{-1} (s, C—H); 1741 (v.s., $-O-CO-CH_3$); 1685 cm^{-1} (s, $-CO_2H$); 1250 and 1240 cm^{-1} (s, $-O-CO-CH_3$).

Mass spectrum: 556 (P, $C_{34}H_{52}O_6$, 36%), 496 ($C_{32}H_{48}O_4$, 9%), 436 ($C_{30}H_{43}O_2$, 30%), 219 ($C_{14}H_{19}O_2$, 34%), 218 ($C_{16}H_{26}$, 100%), 203 ($C_{15}H_{23}$, 79%), 189 ($C_{14}H_{21}$, 75%), 175 ($C_{13}H_{19}$, 43%), 161 ($C_{12}H_{17}$, 33%), 149 ($C_{11}H_{17}$, 35%), 147 ($C_{11}H_{15}$, 47%), 135 ($C_{10}H_{15}$, 91%), 121 (C_9H_{13}, 86%).

U.V. (MeOH) λ_{max} = 210 (ε = 100).

C.D. (C, 1.9 gms/ml, MeOH)

$[\theta]_{380}$ 0; $[\theta]_{342}$ - .234; $[\theta]_{300}$ 0; $[\theta]_{250}$ .409; $[\theta]_{240}$ 0; $[\theta]_{224}$ - 14.63.

Preparation of O,O-diacetyldihydrocyathic acid

Platinum oxide (2 mgms, 0.009 mmoles) was added to a solution of O,O-diacetylcyathic acid (119) (14 mgms, 0.03 mmoles) in methanol (10 mls). The mixture was hydrogenated at room temperature and with constant stirring under 1 atm. of hydrogen for 12 hours. The platinum was removed by filtration and the methanol concentrated *in vacuo* to yield O,O-diacetyldihydrocyathic acid (123). Yield 11.4 mgms (84%). After recrystallization from methanol, needles were obtained M.P. 306-308.

P.M.R. ($CDCl_3$): δ 4.8-4.5 (m, 2H, C<u>H</u>—OAc), δ 2.01 (s, 3H, O—COC<u>H</u>$_3$), δ 1.98 (s, 3H, O—COC<u>H</u>$_3$), δ 1.01 (s, 6H, C<u>H</u>$_3$—CR_3), δ 1.85 (b.s., 12H, C<u>H</u>$_3$—CR_3, C<u>H</u>$_3$—CH), δ 0.70 (s, 3H, CH_3—CR_3).

I.R. ($CHCl_3$, 0.5 mm): 3300-3050 cm^{-1} (b, OH), 2970 cm^{-1} (s, C—H), 1738 cm^{-1} (s, —O—$COCH_3$), 1685 cm^{-1} (s, CO_2H), 1268 cm^{-1} (s, $OCOCH_3$).

Mass spectrum: 558 ($C_{34}H_{54}O_6$, 8%), 498 ($C_{32}H_{50}O_4$, 10%), 438 ($C_{30}H_{46}O_2$, 47%), 395 ($C_{27}H_{39}O_2$, 13%), 220 ($C_{16}H_{28}$, 10%),

219 ($C_{14}H_{19}O_2$, 34%), 218 ($C_{14}H_{18}O_2$, 11%), 191 ($C_{14}H_{23}$, 43%), 149 ($C_{11}H_{17}$, 36%), 135 ($C_{10}H_{15}$, 100%), 123 (C_9H_{15}, 61%), 109 (C_8H_{13}, 41%), 107 (C_8H_{11}, 47%).

Preparation of cyathic acid (119)

Anhydrous sodium carbonate (50 mgms, 0.4 mmoles) was added to a solution of O,O-diacetylcyathic acid (122) (14 mgms, 0.03 mmoles) in methanol (10 mls). The mixture was vigourously stirred at room temperature for 48 hours. When a shorter reaction time was used the product was a mixture of a diol and monoacetate. The methanol solution was concentrated to dryness *in vacuo* and the residue dissolved in a mixture of pH 4 buffer (Fischer 1M, 10 mls) and chloroform (10 mls). The organic layer was removed and the aqueous layer extracted twice more with chloroform. The chloroform extracts were combined, dried over $MgSO_4$, and concentrated *in vacuo* to yield pure cyathic acid (119) 10.8 mgms (92%).
M.P. 307-9 (after sublimation at 280°C).

P.M.R. ($CDCl_3$): δ 4.66 (d, 2H, J = 6 Hz, $C{=}C\underline{H}_2$), δ 3.8-3.0 (m, 2H, $C\underline{H}{-}OH$), δ 1.7 (s, 3H, $C\underline{H}_3{-}C{=}C$), δ 1.05 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.93 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.88 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.72 (s, 6H, $C\underline{H}_3{-}CR_3$).

I.R. (film cast): 3400 cm^{-1} (s, O—H), 2960 cm^{-1} (s, C—H), 1685 cm^{-1} (s, CO_2H).

Mass spectrum: 472 (P, $C_{30}H_{48}O_4$, 65%), 457 ($C_{29}H_{45}O_4$, 21%), 545 ($C_{30}H_{46}O_3$, 19%), 429 ($C_{27}H_{41}O_4$, 34%), 219 ($C_{14}H_{19}O_2$, 14%), 218 ($C_{16}H_{26}$, 67%), 203 ($C_{15}H_{23}$, 77%), 189 ($C_{14}H_{21}$, 74%), 175 ($C_{13}H_{19}$, 50%), 135 ($C_{10}H_{15}$, 83%), 121 (C_9H_{13}, 100%), 107 (C_8H_{11}, 96%), 95 (C_7H_{11}, 94%).

Preparation of O,O-diacetoxymethylcyathate (124)

A solution of diazomethane in CH_2Cl_2 was added dropwise to a solution of O,O-diacetylcyathic acid (42 mgms, 0.08 mmoles) in CH_2Cl_2 (10 mls) until the yellow colour was maintained. The solution was stirred for 12 hours until examination by T.L.C. (silica gel/chloroform/methanol) indicated that the reaction was complete. Concentration of the CH_2Cl_2 solution to dryness under reduced pressure and crystallization of the residue from methanol gave O,O-diacetylmethylcyathate (124), 53.2 mgms (96%).

M.P. 235°C.

P.M.R. ($CDCl_3$): δ 4.7 to 4.4 (m, 4H, C$\underline{H}$—OAc, C=C$\underline{H}_2$), δ 3.70 (s, 3H, O—C$\underline{H}_3$), δ 2.0 (s, 3H, OCOC$\underline{H}_3$), δ 1.95 (s, 3H, OCOC$\underline{H}_3$) δ 1.67 (s, 3H, C$\underline{H}_3$—C=C), δ 0.98 (s, 3H, C$\underline{H}_3$—CR_3), δ 0.85 (S, 3H, CH_3—CR_3), δ 0.82 (s, 6H, CH_3—CR_3), δ 0.69 (s, 3H, CH_3—CR_3).

I.R. (film cast): 2975 cm^{-1} (s, C—H), 1740 cm^{-1} (s, $OCOCH_3$), 1719 cm^{-1} (s, CO_2CH_3), 1250 and 1240 cm^{-1} (v.s., $OCOCH_3$).

Mass spectrum: 570 (P, $C_{35}H_{54}O_6$, 100%), 510 ($C_{33}H_{50}O_4$, 28%), 450 ($C_{31}H_{46}O_2$, 22%), 391 ($C_{29}H_{43}$, 18%), 246 ($C_{16}H_{22}O_2$, 29%), 233 ($C_{15}H_{21}O_2$, 44%), 218 ($C_{16}H_{26}$, 73%), 203 ($C_{15}H_{23}$, 49%), 189 ($C_{14}H_{21}$, 48%), 147 ($C_{11}H_{15}$, 30%), 135 ($C_{10}H_{15}$, 48%), 121 (C_9H_{13}, 52%), 107 (C_8H_{11}, 55%), 93 (C_7H_9, 53%).

Preparation of 1,0-acetoxy-3,0-hydroxymethylcyathate (126)

Anhydrous potassium carbonate (50 mgms, 0.36 mmoles) was added to a solution of 0,0-diacetoxymethylcyathate (124) (20 mgms, 0.035 mmoles) in methanol. The solution was stirred rapidly at room temperature for 30 minutes and then let stand without stirring for 8 hours. The reaction solution was evaporated to dryness and the residue dissolved in a mixture of water (10 mls) and methylene chloride (10 mls). The organic layer was separated and dried over $MgSO_4$. Removal of the methylene chloride under reduced pressure gave 1,0-acetoxy-3,0-hydroxymethylcyathate (126). Yield 16.7 mgms (90%). This was recrystallized from methanol to yield white clusters. M.P. 208-10°C.

P.M.R. ($CDCl_3$): δ 4.61 (d, 2H, J = 8, $C{=}C\underline{H}_2$), δ 4.54 (d of d, 1H, J = 16 & 5, $C\underline{H}{-}OAc$), δ 3.69 (s, 3H, $-OC\underline{H}_3$), δ 3.26 (d of d, 1H, J = 12.5 and 4.5, $C\underline{H}$-OH), δ 1.97 (s, 3H, $C\underline{H}_3{-}CO{-}O$), δ 1.67 (b.s., 3H, $C\underline{H}_3{-}C{=}C$), δ 0.98 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.94 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.83 (s,

3H, $C\underline{H}_3-CR_3$), δ 0.75 (s, 3H, $C\underline{H}_3-CR_3$), δ 0.69 (s, 3H, $CH_3-C\underline{H}_3$).

I.R. (film cast): 3500 cm^{-1} (b.w, OH), 2960 cm^{-1} (s, C—H), 1718 cm^{-1} (s, $-O-COCH_3$), 1250 cm^{-1} (s, $-O-COCH_3$).

Mass spectrum: 528 (P, $C_{33}H_{52}O_5$, 100%), 513 ($C_{32}H_{49}O_5$, 24%), 468 ($C_{31}H_{48}O_3$, 45%), 233 ($C_{15}H_{21}O_2$, 52%), 218 ($C_{16}H_{26}$, 81%), 203 ($C_{15}H_{23}$, 78%), 189 ($C_{14}H_{21}$, 68%), 175 ($C_{13}H_{19}$, 36%), 147 ($C_{11}H_{15}$, 40%), 135 ($C_{10}H_{15}$, 60%), 121 (C_9H_{13}, 75%), 107 (C_8H_{11}, 75%), 95 (C_7H_{11}, 67%), 93 (C_7H_9, 72%).

Preparation of methyl cyathate (125)

Lithium aluminum hydride (20 mgms, 0.53 mmoles) was added to a solution of O,O-diacetylmethylcyathate (124) (20 mgms, 0.035 mmoles) in anhydrous ether (10 mls). The mixture was stirred at room temperature for 24 hours. The reaction was stopped by slowly adding the ether solution to aqueous $NaHCO_3$ solution (20 mls). The ether layer was removed and the aqueous layer extracted twice more with ether. After drying over Na_2SO_4 the ether was concentrated *in vacuo* to yield methyl cyathate (125). Yield 15.7 mgms (92%). The methyl cyathate could be recrystallized from hexane/chloroform to give long needles. M.P. 131-133°C.

P.M.R. ($CDCl_3$): δ 4.52 (d, 2H, J = 9, $C{=}C\underline{H}_2$), δ 3.09 (s, 3H, $-OC\underline{H}_3$), δ 3.44 (d of d, 1H, J = 1 and 5, $C\underline{H}-OH$), δ 3.21 (d of d, 1H, J = 12 and 4.5, $C\underline{H}-OH$), δ 1.68 (b.s., 3H, $C\underline{H}_3-C{=}C$), δ 1.0 (s, 3H, CH_3-CR_3), δ 0.92 (s, 3H, CH_3-CR_3), δ 0.71 (s, 6H, CH_3-CR_3), δ 0.68 (s, 3H, CH_3-CR_3).

I.R. (film cast): 3390 cm^{-1} (s, O—H), 2950 cm^{-1} (s, C—H), 1718 cm^{-1} (s, CO_2CH_3).

Mass spectrum: 486 (P, $C_{31}H_{50}O_4$, 100%), 443 ($C_{28}H_{43}O_4$, 26%), 426 ($C_{29}H_{46}O_2$, 33%), 269 ($C_{15}H_{25}O_4$, 27%), 218 ($C_{16}H_{26}$, 60%), 203 ($C_{15}H_{23}$, 73%), 189 ($C_{14}H_{21}$, 73%), 175 ($C_{13}H_{19}$, 41%), 149 ($C_{11}H_{17}$, 43%), 135 ($C_{10}H_{15}$, 60%), 121 (C_9H_{13}, 82%), 109 (C_8H_{13}, 58%), 107 (C_8H_{11}, 78%), 95 (C_7H_{11}, 76%), 93 (C_7H_9, 77%).

Preparation of methyl-O,O-diacetyldihydrocyathate (127)

A solution of diazomethane in methylene chloride was added slowly to a solution of O,O-diacetyldihydrocyathic acid (123) until the yellow colour was maintained. The solution was stirred for 12 hours at room temperature and then concentrated under reduced pressure to yield methyl-O,O-diacetyldihydrocyathate (127). Yield 10 mgms (98%).

P.M.R. ($CDCl_3$): δ 4.50 (d of d, 1H, J = 11 and 6, C<u>H</u>—OAc), δ 4.40 (d of d, 1H, J = 12 and 5, C<u>H</u>—OAc), δ 3.70 (s, 3H, —OC<u>H</u>$_3$), δ 2.0 (s, 3H, C<u>H</u>$_3$—O—CO), δ 1.97 (s, 3H, C<u>H</u>$_3$—O—CO), δ 0.98 (s, 3H, C<u>H</u>$_3$—CR_3), δ 0.80 (m, 12H, C<u>H</u>$_3$), δ 0.68 (s, 3H, C<u>H</u>$_3$—CR_3).

I.R. (film cast): 2980 cm^{-1} (s, C—H), 1740 cm^{-1} (s, OCO—CH_3), 1720 cm^{-1} (sh, —CO_2CH_3), 1250 and 1240 cm^{-1} (s, $OCOCH_3$).

Mass spectrum: 572 (P, $C_{35}H_{56}O_6$, 35%), 512 ($C_{33}H_{52}O_4$, 25%), 453 ($C_{31}H_{49}O_2$, 30%), 452 ($C_{31}H_{48}O_2$, 84%), 233 ($C_{15}H_{21}O_2$, 61%), 191 ($C_{14}H_{23}$, 61%), 161 ($C_{12}H_{17}$, 25%), 149 ($C_{11}H_{17}$, 44%), 135 ($C_{10}H_{15}$, 100%), 121 (C_9H_{13}, 57%), 109 (C_8H_{13}, 51%), 107 (C_8H_{11}, 65%), 95 (C_7H_{11}, 89%).

<u>Preparation of the diketone (121)</u>

Jones' reagent (8M) was added to a solution of cyathic acid (<u>119</u>) (2 mgms, 0.004 mmoles) in acetone until the orange colour was sustained (1 drop was required). The solution was stirred for 5 minutes and then quenched by the addition of 4 drops of isopropyl alcohol. The reaction mixture was filtered and then concentrated under reduced pressure to give a residue. This residue was dissolved in 2 mls of CH_2Cl_2 and washed with water. After drying the CH_2Cl_2 solution over $MgSO_4$ and concentration *in vacuo* the ketone (<u>121</u>) was obtained. Yield

1.8 mgms (91%). The ketone was recrystallized from methanol as colourless clusters. M.P. 285-6°C.

P.M.R. ($CDCl_3$): δ 4.62 (d, 2H, J = 10, $C{=}CH_2$), δ 3.38 (s, ~ 1H), δ 3.3-2.7 (b, ~ 1H), δ 1.70 (b.s., 3H, $C\underline{H}_3-C{=}C$), δ 1.15 (s, 3H, CH_3-CR_3), δ 1.18 (s, 6H, CH_3-CR_3), δ 1.01 (s, 3H, CH_3-CR_3), δ 0.74 (s, 3H, CH_3-CR_3).

I.R. (film cast): 2760 cm^{-1} (s, C—H), 1720 cm^{-1} (sh, w), 1698 cm^{-1} (s), 1676 cm^{-1} (s).

Mass spectrum: 468 (P, $C_{30}H_{44}O_4$, 100%), 218 ($C_{16}H_{26}$, 32%), 203 ($C_{15}H_{23}$, 56%), 189 ($C_{14}H_{21}$, 51%), 175 ($C_{13}H_{19}$, 22%), 153 ($C_9H_{13}O_2$, 63%), 121 (C_9H_{13}, 49%), 107 (C_8H_{11}, 47%), 95 (C_7H_{11}, 42%), 93 (C_7H_9, 55%).

U.V. (MeOH): λ_{max} (ε = 10,000)
after addition of 1 drop 0.1N NaOH λ_{max} = 288 (ε = 26,600).

Preparation of lupanone (128)

A mixture of glochidone (108) (150 mgms, 0.35 mmoles) and palladium on charcoal (5 mgms) in methanol (25 mls) was hydrogenated with 1 atm. of hydrogen and continuous stirring for eight hours. The mixture was then filtered to remove the palladized charcoal and the filtrate concentrated *in vacuo* to give lupanone (128). Yield 141 mgms (93%). M.P. 203-4°C (lit 203-4 ref 117).

P.M.R. ($CDCl_3$): δ 2.3 (m, 2H, $C\underline{H}_2-CO$), δ 2.0-1.7 (m, 48H).

I.R. (film cast): 2950 cm^{-1} (s, C—H), 1701 cm^{-1} (s, C=O).

Mass spectrum: 426 (P, $C_{30}H_{50}O$, 96%), 411 ($C_{29}H_{47}O$, 25%), 383 ($C_{27}H_{44}O$, 41%), 206 ($C_{15}H_{26}$, 42%), 206 ($C_{14}H_{22}O$, 26%), 205 ($C_{15}H_{25}$, 26%), 205 ($C_{14}H_{21}O$, 100%), 191 ($C_{14}H_{23}$, 49%), 163 ($C_{12}H_{19}$, 63%), 149 ($C_{11}H_{17}$, 51%), 123 (C_9H_{15}, 86%), 121 (C_9H_{13}, 57%), 109 (C_8H_{13}, 64%), 107 (C_8H_{11}, 52%), 95 (C_7H_{11}, 86%), 93 (C_7H_9, 47%).

O.R.D. (E, 6.55 mgms/ml, $CHCl_3$): $[\phi]_{400}$ + 746; $[\phi]_{311}$ + 2,236, $[\phi]_{292}$ 0; $[\phi]_{275}$ - 1,100; $[\phi]_{249}$ 0.

C.D. (C, 6.55 mgms/ml, $CHCl_3$): $[\theta]_{400}$ 0; $[\theta]_{292}$ + 2,276, $[\theta]_{240}$ + 10.

Preparation of lupanol (129)

Sodium borohydride (13 mgms, 0.34 mmoles) was added to a solution of lupanone (128) (140 mgms, 0.33 mmoles) in dry T.H.F. (50 mls). The mixture was stirred for 12 hours and then concentrated under reduced pressure. The residue was redissolved in a mixture of water (20 mls) and methylene chloride (20 mls). The organic layer was removed and the aqueous layer extracted twice more with methylene chloride. The methylene chloride solution

was dried over Na_2SO_4 and concentrated *in vacuo* to yield pure lupanol (129). Yield 119 mgms (85%).

P.M.R. ($CDCl_3$): δ 3.2 (d of d, 1H, J = 10 and 6, C<u>H</u>—OH), δ 1.9-0.6 (m, 51H).

I.R. (film cast): 3380 cm^{-1} (s, O—H), 2950 cm^{-1} (s, C—H).

Mass spectrum: 428 (P, $C_{30}H_{52}O$, 70%), 207 ($C_{14}H_{23}O$, 58%), 189 ($C_{14}H_{21}$, 43%), 135 ($C_{10}H_{15}$, 47%), 123 (C_9H_{15}, 56%), 121 (C_9H_{13}, 48%), 109 (C_5H_{13}, 51%), 95 (C_7H_{11}, 92%), 93 (C_7H_9, 61%), 69 (C_5H_9, 100%).

<u>Preparation of lupeol acetate</u> (<u>130</u>)

Acetyl chloride (39 mgms, 35 µl, 0.5 mmoles) was added to a solution of lupeol (<u>129</u>) (110 mgms, 0.26 mmoles) and pyridine (48 mgms, 49 µl, 0.6 mmoles) in methylene chloride (10 mls). The solution was stirred at room temperature for 1 hour and then diluted with saturated $NaHCO_3$ solution (20 mls). The organic layer was removed and the aqueous layer extracted twice more with methylene chloride. Combination of the organic layers, drying over $MgSO_4$ and concentration under reduced pressure yielded crude lupeol acetate (<u>130</u>). This was treated under high vacuum overnight to remove final traces of pyridine. Yield 109 mgms (90%). This material was recrystallized from chloroform/methanol to yield clear transparent plates M.P. 245-7°C (lit 245-6 Ref 117).

Anal: Calcd C, 81.64 H, 11.56

Found C, 81.78 H, 11.70

P.M.R. ($CDCl_3$): δ 4.50 (d of d, 1H, J = 10 and 6, C<u>H</u>—OAc), δ 2.04 (S, 3H, C<u>H</u>$_3$—CO), δ 2.8-1.6 (m, 50H).

I.R. (film cast): 2960 cm^{-1} (s, C—H), 1730 cm^{-1} (s, $OCOCH_3$), 1250 cm^{-1} (s, $OCOCH_3$).

Mass spectrum: 470 (P, $C_{32}H_{54}O_2$, 40%), 410 ($C_{30}H_{50}$, 30%), 249 ($C_{16}H_{25}O_2$, 28%), 189 ($C_{14}H_{21}$, 100%), 136 ($C_{10}H_{16}$, 54%), ($C_{10}H_{15}$, 50%), 121 (C_9H_{13}, 57%), 109 (C_8H_{13}, 47%), 107 (C_8H_{11}, 46%), 95 (C_7H_{11}, 68%).

<u>Preparation of Glochidonyl acetate (131)</u>

Acetyl chloride (60 mgms, 55 µl, 0.76 mmoles) was added to a solution of glochidonol (<u>109</u>) (180 mgms, 0.41 mmoles) and pyridine (70 mgms, 72 µl, 0.88 mmoles) in methylene chloride (20 mls). The mixture was stirred for 2 hours at room temperature and then diluted with saturated $NaHCO_3$ solution. The organic layer was drawn off and the aqueous layer extracted twice more with methylene chloride. The organic fractions were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was kept under high vacuum overnight to remove final traces of pyridine to give Glochidonol acetate (<u>131</u>). Yield 190 mgms (96%). It was recrystallized from methanol to give needles. M.P. 195-6 (lit 196 Ref 114).

P.M.R. ($CDCl_3$): δ 4.9 (d of d, 1H, J = 8.2 and 3, C<u>H</u>—OAc), δ 4.6 (d, 2H, C=CH_2), δ 3.1 (d of d, 1H, J = 15 and 8.2, AcO—C<u>H</u>—CO), δ 2.1 (d of d, 1H, J = 15 and 3, AcO—CH—CO), δ 2.05 (s, 3H, CH_3—CO—O), δ 1.65 (b.s., 3H, CH_3—C=C), δ 1.6-0.6 (m, 39H).

I.R. (film cast): 2960 cm^{-1} (s, C—H), 1730 cm^{-1} (s, O—$COCH_3$), 1720 cm^{-1} (s, R_2CO), 1240 cm^{-1} (s, O—$COCH_3$).

Mass spectrum: 482 (P, $C_{32}H_{50}O_3$, 15%), 422 ($C_{30}H_{46}O$, 100%), 189 ($C_{14}H_{21}$, 31%), 149 ($C_{11}H_{17}$, 29%), 147 ($C_{11}H_{15}$, 32%), 135 ($C_{10}H_{15}$, 37%), 133 ($C_{10}H_{13}$, 33%), 121 (C_9H_{13}, 64%), 109 (C_8H_{13}, 68%), 107 (C_8H_{11}, 70%), 95 (C_7H_{11}, 72%), 93 (C_7H_9, 67%), 91 (C_7H_7, 32%).

<u>Preparation of 1,β-acetyoxy-3,β-hydroxylupene (132)</u>

Sodium borohydride (15 mgms, 0.4 mmoles) was added to a solution of glochidonol acetate (<u>131</u>) (185 mgms, 0.38 mmoles) in dry T.H.F. (20 mls). The mixture was stirred at room temperature for 24 hours. The T.H.F. was removed under reduced pressure and the residue dissolved in a mixture of saturated $NaHCO_3$ solution (20 mls) and methylene chloride (20 mls). The organic layer was drawn off and the aqueous layer extracted twice more with methylene chloride. The methylene chloride solution was dried over Na_2SO_4 and concentrated *in vacuo* to give 1,β-acetoxy,3,β-hydroxylupene (<u>132</u>). Yield

159 mgms (86%). This was recrystallized from methanol to give white clusters. M.P. 215-217°C.

P.M.R. ($CDCl_3$): δ 4.5 (m, 3H, $C{=}CH_2$, $C\underline{H}{-}OAc$), δ 3.3 (d of d, 1H, J = 14 and 5, $C\underline{H}{-}OH$), δ 2.0 (s, 3H, $C\underline{H}_3{-}CO{-}O$), δ 1.68 (b.s., 3H, $C\underline{H}_3{-}C{=}C$), δ 1.03 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 1.00 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.96 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.92 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.78 (s, 6H, $C\underline{H}_3{-}CR_3$).

I.R. (film cast): 3450 cm^{-1} (m, O—H), 2970 cm^{-1} (s, C—H), 1720 cm^{-1} (s, $OCOCH_3$), 1250 cm^{-1} (s, $OCOCH_3$).

Mass spectrum: 484 (P, $C_{32}H_{52}O_3$, 7%), 424 ($C_{30}H_{48}O$, 37%), 189 ($C_{14}H_{21}$, 21%), 135 ($C_{10}H_{15}$, 22%), 121 (C_9H_{13}, 33%), 109 (C_8H_{13}, 37%), 107 (C_8H_{11}, 33%), 95 (C_7H_{11}, 40%), 93 (C_7H_9, 31%).

Preparation of 1β,3β-diacetoxy lupene (133)

Acetyl chloride (40 mgms, 36 µl, 0.5 mmoles) was added to a solution of 1,β-acetoxy-3,β-hydroxylupene (132) (150 mgms, 0.31 mmoles) and pyridine (47 mgms, 49 µl, 0.6 mmoles) in methylene chloride (20 mls). The solution was stirred at room temperature for 30 minutes and then diluted with an equal volume of saturated $NaHCO_3$ solution. The organic layer was drawn off and the aqueous layer extracted twice with methylene chloride. The methylene chloride layers were combined and dried

over $MgSO_4$ and concentrated *in vacuo* to 1β,3β-diacetoxy lupene (133). Yield 148 mgms (91%). This was recrystallized from methanol to give white clusters. M.P. 208-10°C (lit 208-10 Ref 114).

Anal: Calc. C, 77.52 H, 10.33
Found C, 77.47 H, 10.61

P.M.R. ($CDCl_3$): δ 5.5 (m, 4H, $C{=}CH_2$, $C\underline{H}{-}OAc$), δ 2.0 (s, 3H, $C\underline{H}_3{-}CO{-}O$), δ 1.97 (s, 3H, $C\underline{H}_3{-}CO{-}O$), δ 1.68 (s, 3H, $C\underline{H}_3{-}C{=}C$), δ 1.04 (S, 3H, $C\underline{H}_3{-}Cr_3$), δ 1.03 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.93 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.85 (s, 3H, $C\underline{H}_3{-}CR_3$), δ 0.78 (s, 6H, $C\underline{H}_3{-}CR_3$).

I.R. (film cast): 2970 cm^{-1} (s, C—H), 1737 cm^{-1} (s, $OCOCH_3$), 1250 cm^{-1} (s, $OCOCH_3$).

Mass spectrum: 526 (P, $C_{34}H_{54}O_4$, 19%), 466 ($C_{32}H_{50}O_2$, 54%), 406 ($C_{30}H_{46}$, 29%), 203 ($C_{15}H_{23}$, 43%), 189 ($C_{14}H_{21}$, 66%), 175 ($C_{13}H_{19}$, 32%), 161 ($C_{12}H_{17}$, 33%), 147 ($C_{11}H_{15}$, 45%), 135 ($C_{10}H_{15}$, 60%), 133 ($C_{10}H_{13}$, 45%), 123 (C_9H_{15}, 56%), 121 (C_9H_{13}, 88%), 109 (C_8H_{13}, 85%), 107 (C_8H_{11}, 92%), 95 (C_7H_{11}, 97%), 93 (C_7H_9, 83%), 81 (C_6H_9, 100%).

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